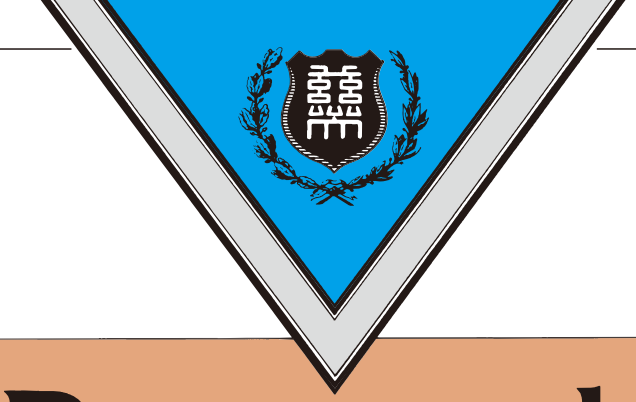

Research Activities

2016

The Jikei University School of Medicine

Research Activities 2016
The Jikei University School of Medicine

Published by
The Jikei University School of Medicine
3-25-8 Nishi-Shimbashi, Minato-ku
Tokyo 105-8461, Japan

December 1, 2017

Contents

Introduction

Research Activities is an annual report of academic achievements at The Jikei University. It was first published in 1989 under the strong leadership of the university's 8th president, Masakazu Abe, who emphasized the importance of keeping a record of the University's activities and sharing that record with the world. Since then, *Research Activities* has been published without interruption for more than a quarter century.

The Jikei University established the Centre for International Affairs in April 2015 to further promote its international activities. The University has sent more than 25 students abroad and received more than one hundred overseas elective students in the academic year 2016. We have partnerships with 10 universities worldwide. A number of researchers are also doing joint research in laboratories all over the world. I hope that *Research Activities* will promote the University's contribution to the global society.

We owe much to the efforts of Professors Naofumi Kimura and Masao Okazaki, and the members of the Academic Information Center in editing this report.

Senya Matsufuji
President
The Jikei University School of Medicine

November 1, 2017

Continuing Medical Education Center
The Continuing Medical Education Committee

Hiroshi Tsuneoka, *Director*
Yasuo Toriumi
Keizo Takagi
Rimei Nishimura

General Summary

The Continuing Medical Education (CME) Center was established in 1982 to commemorate the centennial of The Jikei University and to support the education of doctors outside the university hospital. Registered members consist of alumni throughout Japan, members of the local medical association, and doctors who have been approved by the Jikei CME Center. Members are allowed to use the facilities (video, library) of the Center and other facilities (medical library, medical museum) of the university. A telephone service is available at all times. Members may also attend or participate in summer and monthly seminars sponsored by the Center, and in scientific meetings and conferences held by the department.

Activities

1. Registered members: 193 (as of April 1, 2017)
   Members using the Center: 200/year
2. The 37rd summer seminar was held on August 6, 2016. 72 persons participated.
3. Monthly seminars were held on the second Saturday afternoons of the month in April, May, June, July, September, November, and March. 15 to 25 persons attended each seminar.
4. The “CME Center News” is mailed monthly to the registered members.

Center for Medical Education

Osamu Fukushima, *Professor and Director*
Mariko Nakamura, *Professor*
Hisashi Onoue, *Professor*
Fumiko Okazaki, *Assistant Professor*

General Summary

The Office of Educational Development was founded in 1999. Staff members were recruited from the School of Medicine. Its main interests were (1) the analysis of medical education reports published by the Ministry of Education, Culture, Sports, Science and Technology (MEXT); the Ministry of Health, Labour and Welfare (MHLW); and medical associations; (2) technical support of faculty and management of faculty development and education seminars; and (3) the implementation of tutorials, objective structured clinical examinations, and community-based medical education programs in the undergraduate curriculum. However, many improvements have been required in our undergraduate medical and nursing education, postgraduate clinical training programs, and continuing professional development for healthcare workers. In 2005, the office of Educational Development was reorganized as the Center for Medical Education. Furthermore, the secretariat was set up in the Center in 2006. The bylaw for the Center was revised in 2011, 2013 1nd 2015. The Center now consists of the Branch for Physician Professional Development Support, the Branch for Nursing Professional Development Support, the Branch for Simulation Education, the Branch for Community-based Medical Education and Research, the Branch for Educational Institutional Research and the Branch for Administration. The Branch for Physician Professional Development Support is subdivided into the Office of Undergraduate Medical Education and the Office for Educational Development. The Branches contribute to undergraduate educational activities in medical and nursing schools and practical nursing schools, staff development in the university and 4 attached hospitals, and management of an e-learning system and simulation training centers for students, faculty, and staff in attached hospitals and healthcare providers in the community.

Research Activities

1. Our project "Establishing Systematic Medical Education for Implementing Clinical Clerkship" was given a Supporting Grant for Improving Clinical Clerkship According to a Global Standard for Medical Education Program 2012 by MEXT in 2012. In the activity, Prof. Onoue and Dr. Okazaki engaged in implementation of new clinical practice programs (rotation of all clinical departments for fourth-year and fifth-year students and clinical clerkship rotations for fifth-year and sixth-year). A new type of Post-Clinical Clerkship Objective Structures Clinical Examination (PCC-OSCE) is also preparing as a final OSCE which will implement in next July.
2. Another Supporting Grant for Improving Clinical Clerkship According to a Global Standard for Medical Education Program 2012 was given by MEXT in 2012 to our proj-

ect “Establish an Accreditation System for Basic Medical Education Compliant to Global Standards”. In the collaboration with Tokyo Medical and Dental University, the University of Tokyo, Niigata University, Chiba University and Tokyo Women’s Medical University, we investigated the roles and functions of educational institutional research in medical schools. Educational Institutional Research activity is important for collecting data about educational outcomes in the School of Medicine on carrying on self-evaluation activity in continuing improvement of the educational program. Prof. Fukushima attended to the external evaluation teams as chair at Nippon Medical University, as vice-chair at Juntendo University. Prof. Nakamura joined the external evaluation teams of Juntendo University in the fiscal year.

3. Our proposal “Building of General Practice Capability from Undergraduate to Life-long Learning: To Promote Clinical Research in the Community” was selected by MEXT to receive a Supporting Grant for New Paradigms “Establishing Centers for Fostering Medical Researchers of the Future Application 2013. In this activity, the medical care for the elderly program for third-year students was implemented in March. Now, we implement series of patient contact program starting from first-year to just before entering clinical practice in attached hospitals (forth-year).

4. The Branch for Nursing Professional Development Support organized seminars for education nurses, nurse directors, and nurse administrators.

5. The Office for Educational Development engaged in planning and operation of a forum for medical education leaders (hosted by Japan Medical Education Foundation) and a seminar for medical and dental education leaders (hosted by MEXT).

6. Prof. Fukushima contributed the external evaluation of Jodo Therapy education programs which was supported by MEXT.

7. Prof. Fukushima joined an expert committee (hosted by MEXT) revising the model core curriculum, and to national examination guideline for nurse, health nurse and midwife (hosted by MHLW), and to an action committee (hosted by the Tokyo Metropolis Government) on community health in Tokyo.

8. Contribution to other institutions of higher education (faculty development lectures and workshops): Gifu University, Nara Medical School, Dokkyo Medical School, Niigata University, Hyogo Medical School, IMS Group Patient Safety, Hyogo Medical School Hospital, Aichi Medical School, Kitasato University, Osaka City University, Tokyo Medical Association.

Department of Anatomy (Gross Anatomy and Neuroanatomy)

Yoshinori Kawai, *Professor* Tohru Hashimoto, *Assistant Professor*

General Summary

Our department research activities have focused on neuroanatomy and gross anatomy. In neuroanatomical research, organizations of neuronal networks and the development are investigated to elucidate brain function and diseases using morphological and electrophysiological methods. Our primary interest is focused on quantitative architecture and dynamics of neural circuits and their relationship. In gross anatomical researches, functional importance is explored on variations of organ systems using cadavers and animals.

Research Activities

To integrate and broadcast neural information, local microcircuits and global macrocircuits interact within certain specific nuclei of the central nervous system. The structural and functional architecture of this interaction was addressed for the caudal nucleus of the tractus solitarius (NTS), a relay station of peripheral viscerosensory information processed and conveyed to brain regions concerned with autonomic-affective and other interoceptive reflexive functions.

Geometric and functional architecture of viscerosensory microcircuitry

Is microcircuit wiring designed deterministically or probabilistically? Does geometric architecture predict functional dynamics of a given neuronal microcircuit? These questions were addressed in the viscerosensory microcircuit of the caudal nucleus of the tractus solitaries (NTS), which is generally thought to be homogeneous rather than laminar in cytoarchitecture. Using in situ hybridization histochemistry and whole-cell patch clamp recordings followed by neuronal reconstruction with biocytin filling, anatomical and functional organization of NTS microcircuitry was quantified to determine associative relationships. Morphologic and chemical features of NTS neurons displayed different patterns of process arborization and sub-nuclear localization according to neuronal types: smaller cells featured presynaptic local axons and GABAergic cells were aggregated specifically within the ventral NTS. The results suggested both a laminar organization and a spatial heterogeneity of NTS microcircuit connectivity. Geometric analysis of pre- and postsynaptic axodendritic arbor overlap of reconstructed neurons (according to parent somal distance) confirmed a heterogeneity of microcircuit connectivity that could underlie differential functional dynamics along the dorsoventral axis. Functional dynamics in terms of spontaneous and evoked postsynaptic current patterns behaved in a strongly location-specific manner according to the geometric dimension, suggesting a spatial laminar segregation of neuronal populations: a dorsal group of high excitation and a ventral

group of balanced excitation and inhibition. Recurrent polysynaptic activity was also noted in a subpopulation of the ventral group. Such geometric and functional laminar organization seems to provide the NTS microcircuit with both reverberation capability and a differentiated projection system for appropriate computation of visceral sensory information.

Department of Anatomy (Histology and Embryology)

Masataka Okabe, *Professor*
Hideaki Suzuki, *Assistant Professor*
Hisashi Hashimoto, *Professor*
Yasuyo Shigetani, *Assistant Professor*

General Summary

Our group are interested in the developmental and evolutional aspects of human body structure. By comparing organ development in among vertebrates, we are attempting to reconstitute the evolutionary path that each of our organs has taken, at both the molecular and morphological levels, thus identifying fundamental molecular mechanisms that shape each organ.

Research Activities

Sequencing analysis of the novel hereditary ataxic mouse
We had established a novel hereditary mouse line of neurodegenerative disorder which is transmitted by a single autosomal recessive gene locus *hak*, *hindlimb ataxia with kidney iron deposition*. The affected mouse was characterized by heavy hind limb ataxia with gait disorder, which was firstly recognized at about 4 weeks of age and slowly progressed with advancing age. In this study, sequencing analysis of the hak phenotype was performed to reveal the causative gene locus. Linkage analysis had revealed that the hak phenotype had an association peak on chromosome 2. The most significant association was found in rs13476689 located at 2qE3 (chr2:107,305,044 NCBI37/mm9). Sequencing analysis performed on 101,163,197～111,930,241 in chr 2 revealed many single nucleotide polymorphisms (SNPs), insertions and deletions (indels) of single to oligo nucleotides. Affected mouse specific genetic variations were determined by the following conditions: all affected mice are homozygous and all heterozygotic mice are heterozygous. Known SNPs, which had reference SNP ID numbers, were omitted. In consequence, 892 genetic variations were identified as being hak phenotype specific. None of the genetic variations localized in coding sequences. The genetic locus associated for hak phenotype was mapped to 107,305,356～108,637,615 on chromosome 2qE3, in the vicinity of *bdnf* gene. These results suggest that the hak mouse has a tissue-specific impairment in expression of a type of Bdnf transcripts.

Whole exome analysis of the first case complicated with progressive osseous heteroplasia (POH) and Gorlin syndrome (GS)
In this year, we performed the whole exome analysis (WEA) in the first case complicated with POH and GS, as a collaboration with pediatric department. We detected a nonsense GNAS mutation on the maternal allele and a loss of heterozygosity (LOH) containing GNAS gene on the paternal allele. Interestingly, both mutations were somatic, and probably occurred in early embryonic stage before gastrulation. The first hit might be the nonsense mutation on the maternal allele. Soon after the first hit, the LOH on the paternal

allele might occur by gene conversion with the entire maternal long arm of chromosome 20 containing the GNAS nonsense mutation. As the results, the somatic cells carrying these mutations completely lost the GNAS functions. This somatic mosaicism were observed in several tissues from the patient, such as the peripheral blood lymphocytes, the medulloblastoma, the cardiac myofibroma, dermal myofibroma, and cultured dermal fibroblasts.
Our research suggested that the cause of POH might be somatic mosaicism with complete loss of GNAS function, and that GNAS loss of function mutations might become driver mutations of medulloblastom only when the mutations occurred in early embryonic stage.

Lateral line neuromast in Polypterus *appears superficially during development*
Polypterus, the most basal extant actinopterygian fish in molecular phylogeny like a sarcopterygian Coelacanth, possesses enameled scales on the surface of the body, which reminds us of an extinct primitive actinopterygii or teleostei. Fossil records of these extinct genera reveal no apparent openings on the surface of the scale for the lateral line neuromast, and in the present day a wide variety of shapes are present in the lateral line of existing bony fishes. The lateral line neuromast is the mechano- or chemosensory receptor, which is distributed to the cranial and the lateral body regions from a part of the cranial nerves. All aquatic animals (except for marine mammals) have a neuromast despite the lateral line being considered a vestigial organ for a terrestrial tetrapod that underwent 3 rounds of whole-genome duplication, as did most actinopterygians. We thus investigated the morphogenesis of the lateral line neuromast in *Polypterus* as a representative model of a primitive actinopterygian.
Initial neuromast cells appeared in neurula as the placodes or somewhat later as hair cells expressing *Eya1* or *Sfrp1* to form apparent rosette structures in bistratal epidermis, which was observed in the post-hatch larvae. Adjoining the primitive neuromast, an almost acelluler region was present just under the basement membrane. To examine whether the acellular region is the way of neuromast cells passing through or the space for their axons extending as reported in zebrafish, we are analyzing the acellular region by the Maldi-TOF mass spectrometry. The neuromast along the body existed superficially for life and it did not form a distinct canal organ even after mineralization, implying that the lateral line neuromast in the trunk of *Polypterus* is a superficial type pit organ.

Analysis of origins of lungs and gas bladder using polypterus
Although the lung is an important organ in respiration, it has been a mystery of many researchers for many years when it was acquired during the process of evolution. In recent studies of phylogeny and petrology, it is thought that lungs existed before gas bladder. So we focused on Polypurus to understand which is first lung or gas bladder. Polypterus is a lineage that diverged from the actinopterygian at the earliest stage, and unlike other teleost fish, it has a ventral side air-filled organ (lung) was known. We analyzed lung development in polypterus in detail and revealed that lungs of this fish have very similar developmental mechanism to lung animmal such as mammals. We also found that the expression patterns of genes (*Nkx2.1, Fgf10, Tbx4, Tbx5*) important for lung development were also very similar to mammalian lung development. Furthermore, it was

revealed that the *Tbx4* lung enhancer (LME) sequence, which was known to exist only in the sarcopterygian, was also present on the genome of polypterus and conserved. It was confirmed that this arrangement disappears eventually due to insertion / deficiency, etc. in the teleost. Furthermore, this sequence had functional activites in the lungs of chick embryos. From these experimental results it was found that the ventral air-filled organ of polypterus was homologous to our lungs and the genetic program related to its development was conserved. This finding indicate that the lung development program was existed from a common ancestor of the actinopterygii and the sarcopterygii. This gave us real evidence that the lungs existed before the gas bladder.

Generating transcriptome analysis from the divided diaphragm

Congenital diaphragmatic hernia is disease that causes dysplasia of the diaphragm, but its pathogenesis mechanism is almost unclear. The diaphragm is formed by gathering different surrounding cells where it is formed, but the details of what kind of region the cells form is not clear yet. Understanding the development of diaphragm is very important for understanding the pathogenesis of congenital diaphragmatic hernia and it is therefore indispensable to identify what kind of cell population the diaphragm is formed from. Last year, we divided the diaphragm into six regions and performed transcriptome analysis. As a result, it was revealed that there was a left / right difference in expressing genes depending on the region. Analysis of these candidate genes revealed that there is a difference in gene expression also in PCR using the regional cDNA of the diaphragm. Furthermore, in situ hybridization of these genes revealed that there is site-specific gene expression. It is thought that further clarification of the cell group expressing these genes will contribute to the understanding of congenital diaphragmatic hernia onset in the future.

Joint formation in zebrafish fins

In zebrafish fins, skeletal elements (called as fin rays) are formed by intramembranous ossification. A fin ray consists of multiple segments separated by joints. The fin ray joint morphogenesis is driven by a transcription factor, even-skipped homeobox 1 (evx1), and zebrafish *evx1* mutants exhibit joint agenesis (Schulte et al, 2011). To understand mechanisms of the orderly bone segmentation in the fin ray formation, we have focused on *evx1*-expressing joint cells *in vivo*.

We have generated a transgenic (TG) fish line, which harbored the insertion of the gene construct with the splice-acceptor and Gal4FF in a coding region of *evx1* gene. In this heterozygous TG fish, all fin ray joints were recognized as the green fluorescence protein (GFP)-positive regions by the Gal4-upstream activating sequence (UAS) genetic system. We have found fin ray joints were not formed in homozygous TG fish, and any aggregations of GFP-positive cells were not observed in the fin ray. These data indicate that this TG fish line is evx1-deficient fish caused by the gene trap construct. By using the confocal laser microscope (LSM880, Zeiss), moreover, we have achieved observation of a GFP-positive joint cell with three-dimensional reconstruction. We therefore take advantage of this novel material and method, and try to elucidate spatiotemporal changes of joint cells in the fin ray formation.

Publications

Shigetani Y*[1]*, Wakamatsu Y*[2]*, Tachibana T*[1]*, Okabe M*[1] *(*[1]Jikei Univ,*** **[2]***Tohoku Univ).*** Conversion of neural plate explants to pre-placodal ectoderm-like tissue in vitro. *Biochem Biophys Res Commun.* 2016; **477:** 807-13.

Tatsumi N, Kobayashi R, Yano T, Noda M, Fujimura K, Okada N, Okabe M. Molecular developmental mechanism in polypterid fish provides insight into the origin of vertebrate lungs. *Sci Rep.* 2016; **6:** 30580.

Uchiyama T, Tatsumi N, Kamejima S, Waku T, Ohkido I, Yokoyama K, Yokoo T, Okabe M. "Hypermethylation of the CaSR and VDR genes in the parathyroid glands in chronic kidney disease rats with high-phosphate diet." *Hum Cell.* 2016; **29:** 155-61.

Nagasawa T, Kawaguchi M*[1]*, Yano T, Sano K*[2]*, Okabe M, Yasumasu S*[1] *(*[1]Sophia Univ,*** *[2]***Josai Univ).*** Evolutionary Changes in the Developmental Origin of Hatching Gland Cells in Basal Ray-Finned Fishes. *Zoolog Sci.* 2016; **33:** 272-81.

Miyake T, Kumamoto M, Iwata M, Sato R, Okabe M, Koie H, Kumai N, Fujii K, Matsuzaki K, Nakamura C, Yamauchi S, Yoshida K, Yoshimura K, Komoda A, Uyeno T, Abe Y. The pectoral fin muscles of the coelacanth Latimeria chalumnae; Functional and evolutionary implications for the fin-to-limb transition and subsequent evolution of tetrapods. *Anat Rec.* 2016 Sep; **299:** 1203-23.

Department of Molecular Physiology

Shigeru Takemori, *Professor*
Maki Yamaguchi, *Associate Professor*
Toshiko Yamazawa, *Associate Professor*

General Summary

Our efforts have been concentrated on elucidating mechanisms for achieving biological function through the cooperative interaction of water and proteins.

Research Activities

Differential scanning calorimetry measurement of water components in skinned skeletal muscles

Magnetic resonance images reflect not only water content, but also water states in the tissue. By taking advantage of well-organized skeletal muscle, we have recently clarified that magnetic resonance can be used to distinguish localized water clusters of 5 states. However, the nature of each water state has not been clarified in detail. Interaction between water and macromolecules such as myoproteins in skeletal muscle is considered to restrict their mutual motional freedom. From this, it follows that water and macromolecules would gain additional motional freedom absorbing extra heat with temperature similarly to the melting of ice. With differential scanning calorimetry (DSC), we observed the absorption of extra heat with temperature on skinned fibers. We observed two significant extra heat absorption peaks at $-22^{\circ}C$, $-25^{\circ}C$ and at about the melting point of water. Additionally, we observed two more absorptions peaks at 45˚C and 65˚C in a temperature-dependent irreversible manner. These irreversible heat absorption peaks affected on amplitude of the heat absorptions at $-22^{\circ}C$, $-25^{\circ}C$ independently. Electron microscopy showed that the development of the absorption peak at 45˚C markedly deteriorated A-band structure and that at 65˚C extensively deteriorated sarcomere structure. Therefore, it was found that the peak at 45˚C reflects mainly denaturation of the thick filaments and the peak at 65˚C reflects that of the thin filament. These results suggest that differential scanning calorimetry can be used to effectively explore the water states in sarcomeres.

Property of water around myoprotein studied by quarts crystal microbalance and nuclear magnetic resonance

We observed spin-spin relaxation process of ^{1}H-NMR signals from suspension of myofibrils prepared from rabbit psoas muscle. In the absence of ATP myofibril affects water molecules within 500 nm from its surface differently from water molecules in the bulk solution, and release many water molecules in the presence of ATP.

We also observed the adsorption process of myosin to the gold surface by QCM (quartz crystal microbalance). Viscoelastic property of the myosin adsorbed to the surface of the gold electrode and its surrounding solution as a whole was studied using the AFFINIXQN Pro (Initium, Tokyo).

When myosin adsorption was sparser than 0.2 μg/cm^2, viscoelastic change accompanied by myosin adsorption was almost the same as to the viscoelasticity of buffer without myosin. The resonance frequency falls as does the weight of adsorbed myosin. This implies that myosin adsorbed at low density plays as a solid globular protein. On the other hand, when myosin adsorbed at a higher density, large viscoelastic change has been observed. Viscoelastic analysis indicates that myosin plays as a protein having viscoelasticity, and that ATP binding to the myosin head changes the viscoelasticity of the protein. This suggests that myosins immobilize surrounding solution when it is closely adsorbed. The half of this immobilized solution was released in the presence of ATP or ADP but not in the presence of ATP-γS.
Finally, we observed spin-spin relaxation process of ^{1}H-NMR signals from myofibril suspension in the four major intermediates during the ATP-hydrolysis by myosin. The results implies that the myosin in the M and M.T state immobilized many water molecules, and that myosins in M.D.Pi and M.D states release the water molecules.

Role of polyamines in skeletal muscle hypertrophy
The polyamines putrescine, spermidine and spermine are considered to be essential growth factors in virtually all cells. The proposed roles of polyamines are the functioning of ion channels, nucleic acid packaging, signal transduction, cell proliferation, and differentiation, as well as regulation of gene expression. In skeletal muscle, regulation of polyamine levels is associated with muscle hypertrophy and atrophy, yet the underlying mechanisms of polyamine actions are not well defined. Here, we studied how polyamines may affect the proliferation and/or differentiation of murine myoblast progenitor C2C12 cell line. Upon polyamine treatment of C2C12 cells during induction of myogenic differentiation, the number of myotubes significantly increased. Morphologically, polyamine-treated C2C12 cells exhibited elongated cell body and became multi-nucleated myotubes. On the other hand, the polyamine did not have influence on myoblasts proliferation. Furthermore, compensatory muscle hypertrophy of C57BL6 mice underwent sciatic nerve transection of the left hindlimb was enhanced by administration of polyamines. These results demonstrate that polyamines may play an important role in regulating myogenic differentiation rather than myoblasts proliferation to enhance muscle hypertrophy.

Effect of polyamine on calcium dynamics and electrophysiological property of cardiac cells
Polyamines are poly-cation molecules which are indispensable for proliferation of the eukaryotic cells. On the other hand, polyamines modulate biological functions of ionic channels to modify excitability of the cardiac cells in the physiological condition. Considering these facts, increased polyamine concentration within the cardiac cells may possibly interfere with the function of the ionic channels to induce arrhythmia in athletes who have hypertrophic hearts. To address this issue, intracellular calcium dynamics and electrophysiological property of the cardiac cells were measured *in vitro* and *in vivo* system. Calcium dynamics and electrophysiological property of the isolated cardiac cells were evaluated by fluorescent dyes. Excitability of cardiac cells in the whole body was evaluated by electrocardiograph of the rats under anesthesia. Polyamines increased the duration

of a spontaneous discharge of cardiac cells both *in vitro* and *in vivo*. Polyamine increased intracellular basal calcium concentration in isolated ordinary cardiac cells without corresponding membrane potential change. Amplitude of T-wave of electrocardiograph was increased by the addition of polyamine. Increased intracellular polyamine concentration in cardiac cells may affect hypertrophic hearts of athletes to modify electrophysiological property.

Publications

Sugi H*[1], *Yamaguchi M, Ohno T, Kobayashi T*[2], *Chaen S*[3], *Okuyama H (*[1]*Teikyo Univ,* [2]*Shibaura Inst Tech,* [3]*Nihon Univ). Tension Recovery following Ramp-Shaped Release in High-Ca and Low-Ca Rigor Muscle Fibers: Evidence for the Dynamic State of AMADP Myosin Heads in the Absence of ATP. *PLoS One.* 2016; **11:** 1-14.

Ohno T, Arao T*[1], *Sugi H*[2] *(*[1]*Nihon Univ,* [2]*Teikyo Univ). Effect of Inorganic Phosphate and Low pH on the Force-Velocity Relation of Single Skinned Skeletal Muscle Fibers Studied by Applying Parabolic Fiber Length Changes. *J Nanomed Nanotechnol.* 2017; **8:** 1-5.

Mikami Y*[1,2], *Kanemaru K*[1], *Okubo Y*[1], *Nakaune T*[1], *Suzuki J*[1], *Shibata K*[1], *Sugiyama H*[1], *Koyama R*[1], *Murayama T*[3], *Ito A*[1,4], *Yamazawa T, Ikegaya Y*[1], *Sakurai T*[3], *Saito N*[1], *Kakizawa S*[5], *Iino M*[1,6] *(* [1]*The Univ Tokyo,* [2]*Toho Univ,* [3]*Juntendo Univ,* [4]*Teikyo Univ,* [5]*Kyoto Univ,* [6]*Nihon Univ). Nitric Oxide-induced Activation of the Type 1 Ryanodine Receptor Is Critical for Epileptic Seizure-induced Neuronal Cell Death. *EBioMedicine.* 2016; **11:** 253-61.

Murayama T*[1], *Kurebayashi N*[1], *Ogawa H*[2], *Yamazawa T, Oyamada H*[3], *Suzuki J*[2], *Kanemaru K*[2], *Oguchi K*[2], *Iino M*[2], *Sakurai T*[1] *(*[1]*Juntendo Univ,* [2]*The Univ, Tokyo,* [3]*Showa Univ). Genotype-Phenotype Correlations of Malignant Hyperthermia and Central Core Disease Mutations in the Central Region of the RYR1 Channel. *Hum Mutat.* 2016; **37:** 1231-41.

Yamazawa T, Nakamura N*[1], *Sato M*[2], *Sato C*[2] *(*[1]*Kyoto Univ,* [2]*AIST). Secretory glands and microvascular systems imaged in aqueous solution by atmospheric scanning electron microscopy (ASEM). *Microsc Res Tech.* 2016; **79:** 1179-87.

Reviews and Books

Mikami Y*[1], *Kakizawa S*[2], *Yamazawa T (*[1]*Toho Univ,* [2]*Kyoto Univ). Essential Roles of Natural Products and Gaseous Mediators on Neuronal Cell Death or Survival. *Int J Mol Sci.* 2016; **17:** 1-15.

Department of Cell Physiology

Susumu Minamisawa, *Professor*
Norio Fukuda, *Associate Professor*
Toru Akaike, *Assistant Professor*
Masato Konishi, *Visiting Professor*
Yoichiro Kusakari, *Associate Professor*

General Summary

The aim of research in our laboratory is to understand the regulatory mechanism of the cardiovascular system. In particular, we are interested in the development of the cardiovascular system, the mechanics of sarcomere contraction, Ca^{2+} homeostasis in the cardiac sarcoplasmic reticulum, and the pathophysiology of cardiac fibrosis. We established an experimental system to investigate small fetal arteries, such as the rat fetal ductus arteriosus (DA) and pulmonary vein. In addition, we developed an *in vivo* nanoimaging system to observe sarcomere contraction in the ventricles of small animals, such as rat and mouse.

Research Activities

Molecular mechanism of closure of the DA
The DA is an essential artery that connects the main pulmonary artery and the descending aorta in fetus. The DA closes immediately after birth in accordance with its smooth muscle contraction and vascular remodeling. We are investigating molecular mechanisms of DA closure after birth. The incidence of patent ductus arteriosus (PDA) is known to be higher in premature neonates with infection than in those without infection. However, the detailed mechanism has not been investigated. We found that lipopolysaccharide delays closure of the rat ductus arteriosus by induction of inducible nitric oxide synthase but not prostaglandin E_2.

Causal factors of aortic coarctation
Aortic coarctation is a congenital heart disease whereby the descending aorta is narrow, usually in the area where the DA connects. In some case re-narrowing of the aorta develops after definitive operation. We found that DA smooth muscle cells were straying into aortic smooth muscle cells of narrow area and further extent. We are also investigating the long-term use of prostaglandin E_2 on the human DA. We are collaborating with Hyogo Prefectural Kobe Children's Hospital in this study.

Regulation of sarcoplasmic reticulum ATPase activity
We are interested in regulation of the sarcoplasmic reticulum Ca^{2+}-ATPase and Ca^{2+} homeostasis in the sarcoplasmic reticulum. We found that sarcolipin-knockout mice improved the impairment of muscle function in mdx mice that are an animal model of muscular dystrophy. We are collaborating with National Center of Neurology and Psychiatry in this study.

Regulation of cardiac metabolism

Cardiac metabolism plays an essential role in maintaining cardiac function. Vitamin B1 (VitB1, thiamine) deficiency causes Beriberi, which is characterized by peripheral sensory and motor neuropathy, and congestive heart failure. Dr. Kenehiro Takaki who founded Jikei University, eliminated Beriberi from the Imperial Japanese Navy by improving dietary habit (thiamine supplementation). We found that pretreatment with VitB1 preserved cardiac function in ischemic-reperfusion injury.

Pathophysiological mechanisms of organ fibrosis

Organ fibrosis is a maladaptive response to pathophysiological conditions, such as in impaired organ perfusion and ischemic diseases. However, the effects of pressure-overloaded interstitial fibrosis in the heart and liber in myocardium remain unclear. We prepared pulmonary artery banding (PAB) rats as a model of cardiac hypertrophy. We found that several factors including fibroblast growth factor 23 (FGF23), which is known to play a role in the regulation of osteogenesis, was up-regulated in the interstitial fibrosis group. We also found that low cardiac output was an important determinant that promoted liver fibrosis.

Mechanism of sarcomere contraction in cardiac muscle

1. Simultaneous imaging of local Ca^{2+} and single sarcomere length in rat neonatal cardiomyocytes using yellow Cameleon-Nano140

We developed a novel experimental system for simultaneous nano-imaging of the dynamics of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and single sarcomeres in the subcellular partition, via expression of a FRET-based yellow Cameleon-Nano140 (YC-Nano140) fused into α-actinin for the localization at Z-disks in primary-cultured rat neonatal cardiomyocytes. The system enabled quantitative analyses of local Ca^{2+} transient (CaT) and the ensuing sarcomere dynamics at low and high temperatures during spontaneous beating and at electric stimulation (5 Hz) at 37°C. Local Ca^{2+} waves were observed between CaT, and induced local sarcomeric contractions. There was a positive correlation between an increase in local $[Ca^{2+}]_i$ and the magnitude of sarcomere shortening. This experimental method will be widely applied for elucidating the molecular mechanisms of cardiac excitation-contraction coupling under physiological and pathophysiological conditions (*J. Gen Physiol.*, 2016-2).

2. *In vivo* visualization of sarcomeric motions in the beating mouse heart

The Frank-Starling law predicts that a change in the length of myocardial sarcomeres by only 100 nm dramatically changes the heart's pump functions, indicating the importance of highly accurate measurements of cardiac sarcomere length displacement *in vivo*. In this study, we developed a high speed (100-frames per second), high resolution (20-nm) imaging system for myocardial sarcomeres in living mice. Using this system, we conducted three-dimensional analysis of sarcomere dynamics in left ventricular myocytes during the cardiac cycle, simultaneously with electrocardiogram and left ventricular pressure measurements. We found that (1) the working range of SL was on the shorter end of the resting distribution, and (2) the left ventricular-developed pressure was positively correlated with the SL change between diastole and systole. The present findings provide the

first direct evidence for the tight coupling of sarcomere dynamics and ventricular pump functions in the physiology of the heart (*J. Gen Physiol.*, 2016-1).

3. *In vivo* cardiac excitation-contraction coupling

We developed a novel analysis on the kinetics of Ca^{2+} waves in the mouse heart with the assumption that Ca^{2+} waves expand in a concentric fashion. We found that the velocity of the Ca^{2+} expansion was ～120 μm/s on the confocal *X-Y* plane in a cardiomyocyte, and afterwards, the Ca^{2+} wave propagated at a faster velocity in the longitudinal direction in the myocyte (～170 μm/s). These values were similar to those previously reported in isolated cardiomyocytes. Therefore, our experimental system is usefulness for the analysis of Ca^{2+} waves, and even Ca^{2+} sparks in future studies (*Prog. Biophys. Mol. Biol.* 2017).

Publications

Onda A*[1]*, Kono H*[1]*, Jiao Q*[2]*, Akimoto T*[3]*, Miyamoto T*[4]*, Sawada Y*[5]*, Suzuki K*[2]*, Kusakari Y, Minamisawa S, Fukubayashi T*[6] *(*[1]*Teikyo Univ,* [2]*Hangzhou Normal Univ,* [3]*Tokyo Univ,* [4]*Tsukuba Univ,* [5]*National Rehabilitation Center,* [6]*Waseda Univ). A new mouse model of skeletal muscle atrophy using spiral wire immobilization. *Muscle Nerve.* 2016; **54:** 788-91.

Tsukamoto S, Fujii T, Oyama K, Shintani SA*[1]*, Shimozawa T*[2]*, Kobirumaki-Shimozawa F, Ishiwata S*[2]*, Fukuda N (*[1]*Tokyo Univ,* [2]*Waseda Univ). Simultaneous imaging of local calcium and single sarcomere length in rat neonatal cardiomyocytes using yellow Cameleon-Nano140. *J Gen Physiol*. 2016; **148:** 341-55.

Ito K, Hongo K, Date T, Ikegami M, Hano H, Owada M, Morimoto S, Kashiwagi Y, Katoh D, Yoshino T, Yoshii A, Kimura H, Nagoshi T, Kajimura I, Kusakari Y, Akaike T, Minamisawa S, Ogawa K, Minai K, Ogawa T, Kawai M, Yajima J*[1]*, Matsuo S, Yamane T, Taniguchi I, Morimoto S*[2]*, Yoshimura M (*[1]*The Cardiovascular Institute,* [2]*Kyushu Univ). Tissue thrombin is associated with the pathogenesis of dilated cardiomyopathy. *Int J Cardiol*. 2016; **228:** 821-7.

Kusakari Y, Urashima T, Shimura D, Amemiya E, Miyasaka G, Yokota S, Fujimoto Y, Akaike T, Inoue T, Minamisawa S. Impairment of excitation-contraction coupling in right ventricular hypertrophied muscle with fibrosis induced by pulmonary artery banding. *PLoS One*. 2017; **12:** e0169564. Epub 2017 Jan 9.

Shimozawa T*[1]*, Hirokawa E, Kobirumaki-Shimozawa F, Oyama K, Shintani SA*[2]*, Terui T, Kushida Y, Tsukamoto S, Fujii T, Ishiwata S*[1]*, Fukuda N (*[1]*Waseda Univ,* [2]*Tokyo Univ). In vivo cardiac nano-imaging: A new technology for high-precision analyses of sarcomere dynamics in the heart. *Prog Biophys Mol Biol*. 2017; **124:** 31-40. Epub 2016 Sep 21.

Reviews and Books

Akaike T, Minamisawa S. Prostaglandin E-mediated Vascular Remodeling of the Ductus Arteriosus and Ductus-Dependent Congenital Heart Diseases. *J Mol Genet Med*. 2016; **10:** E109.

Department of Biochemistry

Kiyotsugu Yoshida, *Professor*

General Summary

Tumor is a genetic disease. The fundamental defect of tumor cells is a deregulated proliferation that results from the progressive accumulation of genetic and epigenetic alterations. These alterations invariably affect the regulatory pathways that govern the proper cellular responses to this myriad of signals. Normal proliferative cells are endowed with the abilities to choose between growth and quiescence, differentiation, and apoptosis. The execution of these alternative choices is influenced by physiological factors and stress to achieve a controlled and balanced proliferation. Our research is directed at elucidating signaling pathways that allow normal cells to distinguish between proliferation, differentiation, and apoptosis.

Research Activities

Discovery of the molecular mechanism of metastasis in breast cancer stem cells, iCSCL-10A cells

A line of breast cancer stem cells, iCSCL-10A, was established in 2014 by introducing defined reprogramming factors (OCT4, SOX2, Klf4 and c-Myc) into MCF-10A nontumorigenic mammary epithelial cells. The iCSCL-10A cells possess the hallmarks of cancer stem cells and develop tumors in immunosuppressed mice. However, the metastatic ability of iCSCL-10A cells is unknown. Here, we generated a mouse model of breast cancer bone metastasis. First, we examined, with an in-vivo imaging system, the metastatic ability of iCSCL-10A cells that overexpressed near-infrared fluorescence protein iRFP713 in immunosuppressed mice. Whereas no metastasis developed in mice to which control MCF-10A cells had been injected, bone metastasis near the femur and tibia after 4 weeks in mice to with iCSCL-10A cells had been injected. Furthermore, to investigate the new molecules involved in bone metastasis of iCSCL-10A cells, we isolated metastatic iRFP713-positive iCSCL-10A cells in bone-marrow cell population and analyzed gene expression by microarray. Consequentially, we obtained several genes involved in cell adhesion, signalling, and metabolism. At the present time, we have examined whether these genes function as novel regulators of bone metastasis.

Identification of critical residues required for DYRK2 activity

DYRK2 is an evolutionarily conserved eukaryotic protein kinase that belongs to CMGC protein kinase group. This implies that important structural and functional features are associated with evolutionarily conserved amino acid residues. Among the CMGC kinases, phosphorylation of a regulatory region termed the activation loop is critical to exert their kinase activity. Thus, we attempted to make the series of GFP-fused expression vectors bearing the activation loop mutants of DYRK2. It is known that the kinase activity of

DYRK2 depends on the autophosphorylation of a tyrosine residue (Y382) in activation loop of catalytic domain. Overexpression of wild-type DYRK2 in COS7 cells induced change of its morphology from an elongated epithelial-like shape to a round shape. On the other hand, overexpression of non-phosphorylated mutant, Y382F, remained the epithelial-like morphology and was localized in cytoplasm, but not in nucleus. Predictions using consensus sequences identified the potential phosphorylation sites (Y380, T381 and S385) in DYRK2 activation loop. We also expressed non-phosphorylated mutants (Y380F, T381A and S385A) in COS7 cells. Overexpression of Y380F and T381A exhibited a similar phenotype as the wild-type DYRK2. It was suggested that these residue (Y380 and T381) are dispensable for kinase activity of DYRK2. However, S385A presented the similar phenotype, which was observed in the overexpression of Y382F that has no kinase activity. Furthermore, overexpression of phosphoserine mimic mutants (S385D and S385E) also indicated the similar phenotype that observed in expression of non-phosphorylated mutants, Y382F and S385A. These results may support that the S385 is critical residue required for DYRK2 activity.

Plk1 regulates mitotic chromosome condensation

The chromosomal aberration and genomic instability are hallmarks of cancer. A large proportion of cancer cells is aneuploidy, which contain incorrect number of chromosomes. We have focused on Plk1 that is an essential regulator for proper mitotic progressions and is overexpressed in several cancers. To investigate Plk1 functions in mitosis, aneuploid cancer cell lines were treated with Plk1 inhibitor. Immunoblot analysis revealed that inhibition of Plk1 leads to a reduction of CAP-H2 at mitosis. CAP-H2 is a subunit of condensin II that contributes to mitotic chromosome condensation and segregation. We performed further analysis and revealed that inhibition of Plk1 leads to Cdc20-mediated degradation of CAP-H2. We also demonstrated that Plk1 phosphorylation of CAP-H2 at Ser288 contributes to the stabilization of CAP-H2 and is required for accurate chromosomal condensation during prophase and subsequent chromosomal segregation. These findings suggest that Plk1-mediated phosphorylation controls condensin II functions by modulating CAP-H2 expression levels to control mitotic chromosomal organization.

Pim-1 regulates self-renewal property of colorectal cancer cells by regulating Akt/mTOR pathways

Pim-1 is a proto-oncogenic kinase and involved in several cellular processes including cell survival, cell proliferation and apoptosis. Increased Pim-1 expression is frequently observed in cancer cells and that is correlated with a poor prognosis in various types of cancers. Accumulating evidence has demonstrated that the cancer stem cells (CSCs) are small subpopulation of cancer cells and possess stem-like properties. The sphere culture system is a functional approach to enrich CSCs which including self renewal ability. Although CSCs are associated with the maintenance and growth of tumors, the cellular signaling pathways by which regulates CSCs capacity have not been fully understood. In this study, we show that Pim-1 function is required for self-renewal capacity in colorectal cancer cells. Our results demonstrated that Pim-1 expression is elevated in sphere-forming cells. Depletion of Pim-1 or treatment with the Pim inhibitor SGI-1776 prevented

sphere formation. Furthermore, inhibition of Pim-1 prevented phosphorylation of Akt and ribosomal protein S6 in sphere-forming cells. These findings suggest that Pim-1 could contribute to self-renewal property in colorectal CSCs by maintaining Akt and mTOR signaling.

Publications

Mimoto R, Imawari Y, Hirooka S, Takeyama H, Yoshida K. Impairment of DYRK2 augments stem-like traits by promoting KLF4 expression in breast cancer. *Oncogene.* 2017; **36:** 1862-72.

Mimoto R, Nihira NT, Hirooka S, Takeyama H, Yoshida K. Diminished DYRK2 sensitizes hormone receptor-positive breast cancer to everolimus by inhibiting post-translational degradation of mTOR. *Cancer Lett.* 2017; **384:** 27-38.

Yamamoto T, Nihira NT, Yogosawa S, Aoki K, Takeda H, Sawasaki T, Yoshida K. Interaction between RNF8 and DYRK2 is required for the recruitment of DNA repair molecules to DNA double-strand breaks. *FEBS Lett.* 2017; **591:** 842-53.

Reviews and Books

Dashzeveg N, Yoshida K. Crosstalk between tumor suppressors p53 and PKCδ: Execution of the intrinsic apoptotic pathways. *Cancer Lett.* 2016; **377:** 158-63.

Kagami Y, Yoshida K. The functional role for condensin in the regulation of chromosomal organization during the cell cycle. *Cell Mol Life Sci.* 2016; **73:** 4591-8.

Department of Molecular Biology

Senya Matsufuji, *Professor*
Noriyuki Murai, *Assistant Professor*
Akihiro Oguro, *Assistant Professor*

General Summary

Our research have focused on biological significance of regulating cellular polyamines, in particular through a polyamine-regulating protein "antizyme". Polyamines are ubiquitous biogenic amines that are essential for cell proliferation and related to various phenomena such as differentiation, development, cancer, and autophagy. Three major polyamines, putrescine, spermidine and spermine, are present in mammalian cells. When cellular polyamines increase, AZ is induced through translational frameshift. AZ binds to ornithine decarboxylase (ODC), a key enzyme for polyamine biosynthesis, and inhibits the enzymatic activity and accelerates degradation of the enzyme protein. Thus AZ provides the feedback regulation for the cellular polyamine levels. Mammalian cells express three members of the AZ family (AZ1-3) and each AZ is likely to have specific function.

Research Activities

Interaction between MYCN and AZ2 in neuroblastoma cells

We have previously found that AZ2 interacts with c-Myc and accelerates its degradation in ubiquitin-independent manner. We are interested if AZ2 also interacts with MYCN and regulates its degradation since AZ2 expression is known to correlate with the survival of neuroblastoma patients. We observed using immunofluorescent microscopy that HA-tagged AZ2 (HA-AZ2) colocalizes with endogenous MYCN in the nucleoplasm of neuroblastoma cells, and that a proteasome inhibitor, MG132, shifts the localization to the nucleolus. Knockdown of AZ2 using siRNA stabilized and elevated the level of MYCN in neuroblastoma cell lines. These results suggest that AZ2 regulates MYCN in the neuroblastoma cells as c-Myc.

Analysis of interaction between AZ and ATP citrate lyase

Screening for AZ-binding proteins identified ATP citrate lyase (ACLY), a cytosolic enzyme producing acetyl-CoA that is utilized to synthesize lipid and to acetylate cellular components. We confirmed that both AZ1 and AZ2 bind to ACLY and colocalize with ACLY in the cytoplasm. Unexpectedly, neither AZ1 nor AZ2 accelerated ACLY degradation, like ODC degradation mediated by AZs. Additionally, HA-tagged AZs purified from mammalian cells activated purified ACLY in a dose-dependent manner *in vitro*. Knockdown of AZ1 and/or AZ2 in human cancer cells significantly decreased the ACLY activity as well as cellular levels of acetyl-CoA and cholesterol (Tajima *et al*., 2016).

The effects of high-polyamine diet on metabolites

Polyamines are decreased with aging. To address the effects of ingestion of high-poly-

amine diet, we compared the metabolites between the wild-type mice fed a control diet, the wild-type mice fed a high-putrescine diet, and the Oaz1 (AZ1 gene) heterozygous mice whoes polyamine synthesis especially putrescine synthesis is increased. 132 compounds were detected in the sera of these mice by gas chromatography-mass spectrometry. Two-way ANOVA analysis showed that 25 compounds in comparison between young and old mice and 18 compounds in comparison between control diet, high-putrescine diet and the heterozygous deletion (knockout) of Oaz1 were identified with significant differences for each comparative analysis. Among them, we noted that the level of metabolites pattern of high-putrescine diet mice was similar, to some extent, to that of young mice. We are studying these physiological consequences.

Analysis of AZ +1 ribosomal frameshift mechanism with human in vitro *translation system*

It is known that the termination codon at frameshifting position in AZ mRNA is necessary to induce the +1 ribosomal frameshift. To analyze molecular mechanism of polyamine-induced +1 translational frameshift, we replaced the termination codon (UGA) at shifting position of AZ1 mRNA with sense codons, UUC, UAU or GGA. These constructs were translated in HeLa cell extract *in vitro* translation system. Unexpectedly, +1 framshift was induced by polyamines in all constructs. Mass spectrometry analysis revealed that shifting position of these constructs were the same as the original one. Furthermore, we prepared other constructs replacing the termination codon at shifting position with a leucine codons, CUG, CUA or UUA (codon usage is 4, 0.6 or 0.4%, respectively). Plus 1 framshift was also induced in all these constructs by polyamine and the frameshift efficiency was almost the same among these constructs. These results indicate that ribosome stalling by a codon at frameshifting position is not necessary for AZ +1 frameshift.

Publications

Jaafar ZA*[1], *Oguro A, Nakamura Y*[2], *Kieft JS*[1] (*[1]Univ of Colorado, [2]Univ of Tokyo). Translation initiation by the hepatitis C virus IRES requires eIF1A and ribosomal complex remodeling. *eLife.* 2016; **5:** e21198.

Oguro A, Yanagida A*[1], *Fujieda Y*[1], *Amano R*[1], *Otsu M*[1], *Sakamoto T*[1], *Kawai G*[1], *Matsufuji S* (*[1]Chiba Tech). Two stems with different characteristics and an internal loop in an RNA aptamer contribute to spermine-binding. *J Biochem.* 2017; **161:** 197-206.

Department of Pharmacology

Toshihiko Momiyama, *Professor*
Yuji Ohno, *Assistant Professor*
Taro Ishikawa, *Assistant Professor*
Yukihiro Nakamura, *Assistant Professor*
Naofumi Kimura, *Professor*
Haruhisa Nishi, *Assistant Professor*
Masahito Kawamura, *Assistant Professor*

General Summary

The research interests of the Department of Pharmacology include:
1. Synaptic transmission and its modulation in the basal ganglia and basal forebrain (Toshihiko Momiyama)
2. Neural control of breathing in aquatic vertebrates (Naofumi Kimura)
3. Peripheral benzodiazepine receptors on adrenal cells (Yuji Ohno)
4. Living environments may exacerbate allergic stimulation (Haruhisa Nishi)
5. Analysis of the cerebro-cerebellar interaction using optogenetics (Taro Ishikawa and Misa Shimuta)
6. The basic mechanism underlying anticonvulsant effects of ketogenic diet (Masahito Kawamura)
7. Coupling distance between presynaptic Ca^{2+} channels and synaptic vesicles (Yukihiro Nakamura)
8. Cholinergic modulation of central synaptic transmission (Etsuko Suzuki)

Research Activities

Synaptic transmission and its modulation in the basal ganglia and basal forebrain
Electrophysiological studies using slice patch-clamp recording techniques were performed to analyze synaptic transmission and its modulation by neuromodulators, such as dopamine and serotonin, and their developmental changes in the nigrostriatal or mesolimbic dopaminergic system and in the cholinergic system of the basal forebrain. These systems are involved in various psychological functions as well as their disorders, including Parkinson's disease and Alzheimer's disease. Furthermore, optogenetic activation techniques for neurones in these brain areas have been introduced to analyze neuron type-specific synaptic transmission as well as its modulation. These basic analyses can lead to the identification of the mechanisms underlying the related disorders mentioned above, as well as to the development of novel therapeutic tools.

Neural control of breathing in aquatic vertebrates
The neural respiratory output of the isolated brainstem of *Xenopus laevis* displayed two motor patterns, the lung ventilation-like large bursts and the functionally unidentified small bursts. The lung ventilation-like bursts were abolished by bath application of the low concentration (0.1 μM) of μ-opioid receptor agonist, DAMGO and restored by 1-5 μM naloxone. While, the small bursts were resistant to the low concentration of DAMGO. The small bursts might have a common origin with the buccal rhythm of terrestrial frogs.

Peripheral benzodiazepine receptors on adrenal cells
Peripheral benzodiazepine receptor (PBR) localizes in the outer mitochondrial membrane and not only transfer cholesterol in steroidogenic organs under physiological conditions but also is readily upregulated under various pathological conditions such as cancer, inflammation and neurological disease. We would like to investigate whether endozepine and its metabolite, which we prepared from bovine adrenocortical cells, could be related to these pathological conditions.

Living environments may exacerbate allergic stimulation
Environmental exacerbation of allergic stimuli was investigated using a human mast cell-derived cell line as a screening tool. The results revealed that even non-allergen-composed materials could indirectly enhance allergic-induced degranulation. This enhancement was the result of excessive PI3K activation. These results demonstrate that environmental objects may enhance allergic symptoms in patients with type I allergies. This study was supported by the LIXIL JS Foundation and the reports of the study had been presented on the web site of the LIXIL JS Foundation.

Analysis of the cerebro-cerebellar interaction using optogenetics
The cerebro-cerebellar communication is important in a wide range of brain function including sensory information processing. We investigated the somatosensory signaling pathways to the cerebellar cortex, using transgenic mice whose cerebral cortex can be suppressed by light illumination, and revealed that the direct signals from the trigeminal nucleus and the indirect signals via the somatosensory cortex are integrated not only in the Purkinje cells but also in the granule cells in the cerebellar cortex. In addition, in collaboration with a group of Edinburgh University, we showed that the balance of excitatory and inhibitory synaptic inputs are crucial in action potential generation in Purkinje cells and that disturbance of this balance results in a disorder of locomotive activity of mice.

The basic mechanism underlying anticonvulsant effects of ketogenic diet
A ketogenic diet has been used successfully to treat medically-refractory epilepsy. The mechanisms underlying the success of ketogenic diet therapy, however, are not well understood. We fed rats a ketogenic diet, prepared hippocampal slices, and performed electrophysiology in the seizure-prone CA3 region. Slices from ketogenic diet-fed animals showed reduced excitability, and the effects of the ketogenic diet could be reversed with blockers of adenosine A_1 receptors. These results suggest that the reduction of neuronal activity through activation of adenosine A_1 receptor is one of the key mechanisms underlying anticonvulsant effects of ketogenic diet.

Coupling distance between presynaptic Ca^{2+} channels and synaptic vesicles
Coupling distance between voltage-gated Ca^{2+} channels and synaptic vesicles critically determines the probability and timing of neurotransmitter release. Although the coupling distance has been estimated based on the inhibition of transmitter release by Ca^{2+} chelator EGTA, other presynaptic factors other than coupling distance can affect the EGTA effect.

My simulations of buffered Ca^{2+} diffusion and transmitter release revealed that the inhibitory effect of EGTA is potentiated for a brief Ca^{2+} influx like action potential-induced Ca^{2+} elevation. Time course of presynaptic Ca^{2+} influx is mandatory biophysical parameters to estimate the coupling distance using EGTA.

Cholinergic modulation of central synaptic transmission

Acetylcholine is known to be a neurotransmitter involved in learning and memory. In the central nervous system, several studies has reported that synaptic transmission and firing property of neurons are modulated by acetylcholine. We elucidated the cholinergic modulation in striatum and hippocampus using electrophysiological technique. In the striatum, we have found that GABA release onto cholinergic interneurons is inhibited by activation of muscarine M1 receptors.

Publications

Nishijo T, Momiyama T. Serotonin 5-HT1B receptor-mediated calcium influx-independent presynaptic inhibition of GABA release onto rat basal forebrain cholinergic neurons. *Eur J Neurosci.* 2016; **44:** 1747-60.

Hamada N*[1]*, Ito H*[1]*, Nishijo T, Iwamoto I*[1]*, Morishita R*[1]*, Tabata H*[1]*, Momiyama T, Nagata K*[1] *(*[1]Aichi Human Service Center).*** Essential role of the nuclear isoform of RBFOX1, a candidate gene for autism spectrum disorders, in the brain development. *Sci Rep.* 2016; **6:** 30805.

Nishi H, Pelleg A*[1]*, Schulman ES*[1] *(*[1]Drexel Univ).*** IgE Receptor-Mediated Histamine Release in Human Lung Mast Cells: Modulation by Purinergic Receptor Ligands. *Ann Clin Lab Sci.* 2016; **46:** 463-9.

Kawamura MJ, Ruskin DN*[1]*, Masino SA*[1] *(*[1]Trinity Coll).*** Metabolic Therapy for Temporal Lobe Epilepsy in a Dish: Investigating Mechanisms of Ketogenic Diet using Electrophysiological Recordings in Hippocampal Slices. *Front Mol Neurosci.* 2016; **9:** 112.

Jelitai M*[1]*, Puggioni P*[1]*, Ishikawa T, Rinaldi A*[1]*, Duguid I*[1] *(*[1]Univ Edinburgh).*** Dendritic excitation-inhibition balance shapes cerebellar output during motor behavior. *Nat Commun.* 2016; **7:** 13722.

Reviews and Books

Yamada K*[1]*, Takahashi S*[2]*, Karube F*[2]*, Fujiyama F*[2]*, Kobayashi K*[3]*, Nishi A*[4]*, Momiyama T (*[1]Hirosaki Univ,*** [2]***Doshisha Univ,*** [3]***Fukushima Med Univ,*** [4]***Kurume Univ).*** Neuronal circuits and physiological roles of the basal ganglia in terms of transmitters, receptors and related disorders. *J Physiol Sci.* 2016; **66:** 435-46.

Kawamura M, Jr. Chapter 21, Ketogenic diet in a hippocampal slice: models and mechanisms. In: Ketogenic Diet and Metabolic Therapies: Expanded Roles in Health and Disease. Oxford: Oxford University Press; 2016. p. 186-95.

Department of Pathology

Masahiro Ikegami, *Professor*
Masafumi Suzuki, *Professor*
Satoru Chiba, *Associate Professor*
Koichi Nomura, *Associate Professor*
Tohru Harada, *Assistant Professor*
Yasuhiko Endo, *Assistant Professor*
Akihiko Sakata, *Professor*
Takako Kiyokawa, *Professor*
Hiroyuki Takahashi, *Associate Professor*
Sigeharu Hamatani, *Associate Professor*
Tomoe Ru, *Assistant Professor*
Kazumasa Komine, *Assistant Professor*

General Summary

The objective of our research in the Department of Pathology is to morphologically investigate the causes of disease and to evaluate morphological changes. We used human tissue samples resected at autopsy and surgery or obtained at biopsy. These samples were examined by means such as light microscopy, electron microscopy, morphological measurement, immunohistochemical staining, and molecular pathological techniques.

Outline of Education and Research

Research on the gastrointestinal tract

1. We evaluated the clinicopathological characteristics of early-stage epithelial tumors of the duodenum. The study group comprised 101 patients with intramucosal tumors (110 lesions) whose duodenal epithelial tumors were endoscopically or surgically resected. The mucin phenotype of the tumor was evaluated immunohistochemically, and the histological grade of atypia was classified according to the 2010 World Health Organization classification. In addition, the frequencies of differentiation into fundic glands in the tumors and the underlying gastric mucosa were studied immunohistochemically. The tumors were classified into 2 subgroups according to mucin phenotype: intestinal-type tumors (98 lesions, 89.1%) and gastric-type tumors (12 lesions, 10.9%). The intestinal-type tumors were subclassified into tubular type (91 lesions, 82.7%) and tubulovillous type (7 lesions, 6.4%). Gastric-type tumors were subclassified into gastric foveolar type (3 lesions, 2.7%) and pyloric type (9 lesions, 8.2%) according to mucin phenotype. The grade of atypia was significantly higher in gastric-type tumors. Pyloric gland-type tumors were characterized by the proliferation of mucous glands similar to pyloric glands and showed a high frequency of differentiation into fundic glands. Fundic gland tissue was found in 16 (14.5%) of 110 specimens of non-tumorous (normal) mucosa situated adjacent to lesions. The majority of duodenal epithelial tumors were evaluated to be intestinal-type tumors on the basis of mucin phenotype. However, about 10% of the duodenal epithelial tumors were gastric-type tumors associated with gastric-type mucin. These gastric-type tumors were large lesions with a high histological grade of atypia. Among the gastric-type tumors, 9 lesions (8.2%) were pyloric gland-type tumors, showing a high frequency of differentiation into fundic glands. The presence of fundic glands in the normal mucosa of duodenal tumors suggested that proliferating cells situated in the duodenum possess the ability to differentiate into fundic glands and participate in the develop-

ment of pyloric gland-type tumors.
2. Active inflammation associated with ulcerative colitis (UC) is generally histologically evaluated according to Matts' classification. However, there is often a divergence between Matts' classification and endoscopic findings. We analyzed biopsy specimens and studied the correlation between disease activity evaluated on the basis of endoscopic findings and disease activity evaluated on the basis of histologic findings. The study group comprised 191 patients (527 biopsy specimens) with UC who underwent lower gastrointestinal endoscopy with biopsy in our hospital from June 2015 through June 2016. Six endoscopic findings in the biopsy specimens (non-inflamed mucosa, loss of visible vascular patterns, granular mucosa, friable mucosa, spontaneous bleeding, and erosions and ulcers) were evaluated according to the main variables of Matts' classification and the Mayo classification. Histological findings of the biopsy specimens were evaluated on the basis of the presence or absence of the following 5 findings: the 4 main variables of Matts' classification (neutrophilic infiltration, cryptitis, crypt abscess, and erosions or ulcers) and the presence or absence of basal plasmacytosis (BP). In patients with highly inflamed mucosa associated with endoscopic findings of erosions or ulcers, the rates of histological findings of crypt abscess and of erosions or ulcers were 27.4% and 18.3%, respectively. However, BP was found in 58.5% of patients. BP was thus suggested to be a useful finding suggesting the presence of active inflammation.
3. Six cases of large villous adenoma (lesions consisting of ≥80% villous components) were selected from among the lesions that were resected surgically and endoscopically. Guanine nucleotide binding protein, alpha stimulating gene (GNAS) mutations in the villous adenomas were analyzed by real-time polymerase chain reaction (RT-PCR). The expression of GNAS mutations (601C>T) was confirmed in 4 cases. The villous gland fields obtained from these 4 cases were classified into 2 regions (high-grade atypia region, low-grade atypia region), and 3 sites including regions of non-villous glands at the tumor margin were analyzed. The experimental results suggested that GNAS mutations in villous adenomas show not only homogeneous expression, but also heterogeneous expression.
4. Serrated lesions of the colorectum, for which the diagnostic criteria remain unclear, were studied morphometrically. The lesions were classified on the basis of the maturity of crypt surface cells and the presence or absence of branched tubules. In addition, Ki-67 immunostaining was performed to compare the relations to zones of proliferating cells. Types 1 and 2 corresponded to conventional hyperplastic polyps, and the proliferative zone was located in the crypt floor. Type 3 corresponded to sessile serrated adenomas/polyps, and the proliferative zone extended to the middle of the crypts. Types 4 and 5 corresponded to serrated adenomas, and proliferating cells tended to be distributed in all layers of the crypts.

Research on the liver
1. Patients with autoimmune hepatitis (AIH) underwent several biopsies to study whether histopathological findings such as interface hepatitis, activity findings associated with intralobular focal necrosis, and the status of fibrosis correlate with changes in biochemical data. The results showed that biochemical data such as ALT correlated to some extent

with inflammatory activity, but no correlations were obtained for the status of fibrosis. Therefore, the status of AIH can be ascertained to some extent on the basis of biochemical data, whereas an invasive liver biopsy must be performed to assess the status of fibrosis.

Research on the kidney

1. Molecular pathological studies related to glomerular lesions and tonsillitis in IgA nephropathy: Total RNA in frozen specimens of tonsils from patients with IgA nephropathy (5 specimens) and in tonsils affected by chronic tonsillitis (4 specimens) was amplified by quantitative RT-PCR, and Human Genome U133 Plus 2.0 Array (Affymetrix, Inc.) was used to perform transcriptome analysis (38,500 genes). Genes that showed RNA expression patterns in the tonsils of patients with IgA nephropathy that differed from the RNA expression patterns in the tonsils affected by chronic tonsillitis in the control group were identified, and 17 genes related to conditions such as cancer and lymphoma were focused on. Genes related to increased numbers of T-cell nodules in the tonsils and genes related to the bipolarization of the reticulation of the crypt epithelium were investigated, and a total of 167 genes were identified. In association with chronic stimulation of the tonsils in patients with IgA nephropathy, toll-like receptor 9 induces the overexpression of a proliferation-inducing ligand (APRIL), a member of the tumor necrosis factor (TNF) family, via B cells located primarily in lymph follicles of the tonsils and thereby contributes to the overproduction of galactose-deficient IgA1 (Muto M et al. JASN 2017). In addition, thymic stromal lymphopoietin (TSLP) expressed by dendritic cells of lymph follicles has been reported to promote IgA class switching by increasing IgA plasma cells (Meng H, et al. Transl Res. 2016).

2. Three-dimensional structure of glomerulonephritis on scanning electron microscopy (SEM): Glomeruli of 14 patients with glomerulonephritis were photographed on serial block-face scanning electron microscopy(SBE) and SEM.

In patients with IgA nephropathy (4 patients with acute active disease and 2 patients with chronic advanced disease and lupus nephritis), the cellular composition was colored and segmentation was performed to analyze the 3-dimensional structure. First, in IgA nephropathy and lupus nephritis, podocytes were found to penetrate the glomerular basement membrane and impact the mesangial matrix; contact with mesangial cells was confirmed. On the other hand, mesangial cells were found to enter the subendothelial space under the under the lamina densa of the glomerular basement membrane, and the findings suggested an increase in the mesangial matrix. To our knowledge, this is the first time in the world to demonstrate contact between podocytes and mesangial cells at the time of infolding into the mesangial matrix. Second, when we followed up whether the destruction of the glomerular basement membrane occurred in association with segmental thin basement membrane lesions, podocytic invasion into the intraglomerular space beside the endothelium was confirmed in the glomerular basement membrane in the region near the mesangial matrix. It has previously been suggested that some of the cells considered to be endothelial cells are podocytes.

Research on the urogenital system

1. The urothelial cancers underwent immunostaining (HER2, CK5/6, CK20, CD44) and HER2 fluorescence in situ hybridization (FISH). On FISH 17% of the patients had HER2 IHC 3+ tumors, and gene amplification was found in 13 patients with IHC 2+ tumors. Most of the patients with HER2-positive tumors had luminal-type tumors.
2. Since last year, we have studied the anatomic, developmental, and clinicopathological characteristics of prostate cancer. In 2016, intraductal carcinoma of the prostate (IDC-P) and its outcomes were mainly studied. The incidence of IDC-P is lower in transitional zone cancer than in peripheral zone cancer, and relatively good outcomes will most likely be obtained hereafter. The presence of IDC-P was a significant prognostic factor for poor outcomes in patients who underwent total prostatectomy.

Research on gynecologic pathology

1. Most cases of high-grade serous carcinoma (HGSC) of the female genital organs are serous tubal intraepithelial carcinomas (STIC) arising in the fallopian tubes including the fimbriae and causing infiltrating cancer, metastases to the ovary, and peritoneal seeding. We investigated the incidence of STIC and studied the pathological findings according to the type of primary HGSC (fallopian tube, ovary, endometrium, peritoneum).

Research on molecular pathology

1. To investigate the locations of disease genes related to the development of lung cancer, we studied a total of 306 patients with adenocarcinomas, squamous-cell carcinomas, and neuroendocrine tumors. Microsatellite instability (MSI) analysis was performed 19 DNA markers on chromosome 8p. The rate of MSI at 8p23.2, 8p23.1, 8p22, and 8p21 was 20%, 51%, 24%, and 15%, respectively. The rate of MSI at 8p23.1 was significantly higher than that at the other regions. In particular, a high frequency of MSI was found at the D8S1819 locus for every tissue type, suggesting that a disease gene related to the development of lung cancer is situated at 8p23.1.

Publications

Carleton C, Hoang L, Sah S, Kiyokawa T, Karamurzin YS, Talia KL, Park KJ, MsCluggage WG. A detailed immunohistochemical analysis of a large series of cervical and vaginal gastric-type adenocarcinomas. *Surg pathol.* 2016; **40:** 636-44.

Miki K, Sasaki H, Kido M, Takahashi H, Aoki M, Egawa S. A comparative study on the efficacies of gonadotropin-releasing hormone (GnRH) agonist and GnRH antagonist in neoadjuvant androgen deprivation therapy combined with transperineal prostate brachytherapy for localized prostate cancer. *BMC cancer.* 2016; **16:** 708.

Kimura Y, Kudo S, Miyachi H, Ichimasa K, Kouyama Y, Misawa M, Sato Y, Matsudaira S, Oikawa H, Hisayuki T, Mori Y, Kudo T, Ogata N, Kodama K, Wakamura K, Hayashi T, Katagiri A, Baba T, Hidaka E, Ishida F, Hamatani S. Head invasion is not a metastasis free condition in pedunculated T1 colorectal carcinomas based on the precise histopathological assessment. *Digestion.* 2016; **61:** 166-75.

Watanabe T, Ajioka Y, Mitsuyama K, Watanabe K, Hanai H, Nakase H, Kunisaki R, Matsuda K, Iwakiri R, Hida N, Tanaka S, Takeuchi Y, Ohtsuka K, Murakami K, Kobayashi K, Iwao Y, Nagahori M, Iizuka B, Hata K, Ikegami M, Hirata I, Kudo SE, Matsumoto T, Ueno F, Watanabe G, Ito Y, Oba K, Inoue E, Tomotsugu N, Takabayashi T, Sugihara K, Suzuki Y, Watanabe M, Hibi T. Comparison of targeted vs random biopsies for surveillance of ulcerative colitis-associated colorectal cancer. *Gastroenterology.* 2016; **151:** 1122-30.

Ito K, Hongo K, Date T, Ikegami M, Hano H, Owada M, Morimoto S, Kashiwagi Y, Katoh D,

Yoshino A, Kimura H, Nagoshi T, Kajimura I, Kusakari Y, Akaike T, Minamisawa S, Ogawa K, Minai K, Ogawa T, Kawai M, Yahima J, Matsuno S, Yamane T, Taniguchi I, Morimoto S, Yoshimura M. Tissue thrombin is associated with the pathogenesis of dilated cardiomyopathy. *Int J Cardiol.* 2016; **228:** 821–7.

Kimura T, Takahashi H, Okayasu M, Kido M, Inaba H, Kuruma H, Yamamoto T, Furusato B, Furusato M, Wada T, Egawa S. Time trends in histological features of latent prostate cancer in japan. *J Urol.* 2016; **195:** 1415–20.

Shimura E, Hama T, Suda T, Ikegami M, Urashima M, Kojima H. The presence of HPV DNA in neck lymph node metastasis correlates with improved overall survival of patients with oropharyngeal cancer undergoing surgical treatment. *Oncology.* 2016; **92:** 87–93.

Sato S, Takahashi H, Kimura T, Egawa S, Furusato B, Ikegami M. Clinicopathological importance of anterior prostate cancer in Japanese Men. *Pathol Int.* 2016; **67:** 156–62.

Dobashi A, Goda K, Yoshimura N, Ohya TR, Kato M, Sumiyama K, Matsushima M, Hirooka S, Ikegami M, Tajiri H. Simplified criteria for diagnosing superficial esophageal squamous neoplasms using Narrow Band Imaging magnifying endoscopy. *World J Gastroenterol.* 2016; **22:** 9196–204.

Department of Virology

Kazuhiro Kondo, *Professor* Nobuyuki Kobayashi, *Assistant Professor*

General Summary

Human herpesvirus is capable of establishing a lifelong latent infection of their host, is reactivated frequently. We are studying the molecular mechanism of latency and pathogenesis of human cytomegalovirus (HCMV) and human herpesvirus 6 (HHV-6), and find a novel latent protein of HHV-6 which associate with and mood disorders. We are also trying to apply HHV-6 and HHV-7 to the tools for studying the mechanism of fatigue. Salivary HHV-6 and HHV-7 DNA amounts increased with training and decreased with rest, suggesting usefulness as biomarkers of physiological fatigue. Additionally we study on cognitive impairment and Alzheimer's disease which we have previously shown the relationship to fatigue and herpesvirus reactivation.

Research Activities

HHV-6 and HHV-7 are biomarkers for fatigue which distinguish between physiological fatigue and pathological fatigue

Fatigue reduces productivity and is a risk factor for lifestyle diseases and mental disorders. Physiological fatigue occurs in everyone but decreases with rest. Pathological fatigue, however, greatly reduces quality of life and requires therapeutic interventions. Therefore, these 2 types of fatigue must be distinguished, but biomarkers for distinquishing them have not been identified. We report on the measurement of salivary HHV-6 and HHV-7 as biomarkers for quantifying physiological fatigue. We observed that salivary HHV-6 and HHV-7 increased with military training and work and rapidly decreased with rest. Our results suggest that macrophage activation and differentiation are necessary for virus reactivation. However, HHV-6 and HHV-7 did not increase in subjects with obstructive sleep apnea syndrome, CFS, and major depressive disorder, which are thought to cause pathological fatigue. Thus, HHV-6 and HHV-7 would be useful biomarkers for distinguishing between physiological fatigue and pathological fatigue. Our findings suggest a fundamentally new approach to evaluating fatigue and preventing fatigue-related diseases.

Caregiver burden and fatigue in caregivers of people with dementia: Measuring human HHV-6 and HHV-7 DNA levels in saliva

Purpose: We examined chronic fatigue, which has not been investigated in detail, in caregivers for family members with dementia.

Methods: The subjects of this study were 44 community-dwelling family caregivers and 50 elderly persons who were not caregivers. We measured salivary levels of HHV-6 and HHV-7 DNA and used the Chalder Fatigue Questionnaire (CFQ) to assess levels of fatigue; we also used the Center for Epidemiologic Studies Depression Scale, the Physi-

cal Activity Scale for the Elderly, the Zarit Caregiver Burden Interview, the Mini-Mental State Examination, the Assessment of Motor and Process Skills, and the Dementia Behavior Disturbance Scale.

Results: The salivary HHV-6 DNA levels and the CFQ scores were significantly higher in caregivers than in elderly persons. The salivary HHV-6 DNA levels in caregivers were significantly correlated with depressive symptoms, the cognitive function of the family members with dementia, and the activities of daily living/instrumental activities of daily living abilities of the patients. The CFQ scores in caregivers significantly correlated with caregiver burden, depression symptoms, leisure physical activity, the number of other family caregivers, the hours spent by caregiving per week, behavior disturbances, and activities of daily living/instrumental activities of daily living abilities.

Conclusions: The salivary HHV-6 DNA level is a new biomarker for caregiver exhaustion. To estimate the burden of caregivers of family members with dementia, fatigue assessments should be performed with a questionnaire, such as the CFQ, and the search for a biomarker, such as the salivary HHV-6 DNA level.

Publications

Suzuki A, Shibata N, Kasanuki K, Nagata T, Shinagawa S, Kobayashi N, Ohnuma T, Takeshita Y, Kawai E, Takayama T, Nishioka K, Motoi Y, Hattori N, Nakayama K, Yamada H, Arai H. Genetic Association between Presenilin 2 Polymorphisms and Alzheimer's Disease and Dementia of Lewy Body Type in a Japanese Population. *Dement geriatr cogn dis extra.* 2016; **6:** 90-7.

Osaki T, Morikawa T, Kajita H, Kobayashi N, Kondo K, Maeda K. Caregiver burden and fatigue in caregivers of people with dementia: Measuring human herpesvirus (HHV)-6 and -7 DNA levels in saliva. *Arch Gerontol Geriatr.* 2016; **66:** 42-8.

Shinagawa S, Kobayashi N, Nagata T, Kusaka A, Yamada H, Kondo K, Nakayama K. DNA methylation in the NCAPH2/LMF2 promoter region is associated with hippocampal atrophy in Alzheimer's disease and amnesic mild cognitive impairment patients. *Neurosci Lett.* 2016; **629:** 33-7.

Aoki R, Kobayashi N, Suzuki G, Kuratsune H, Shimada K, Oka N, Takahashi M, Yamadera W, Iwashita M, Tokuno S, Nibuya M, Tanichi M, Mukai Y, Mitani K, Kondo K, Ito H, Nakayama K. Human herpesvirus 6 and 7 are biomarkers for fatigue, which distinguish between physiological fatigue and pathological fatigue. *Biochem Biophys Res Commun.* 2016; **478:** 424-30.

Takeshita Y, Shibata N, Kasanuki K, Nagata T, Shinagawa S, Kobayashi N, Ohnuma T, Suzuki A, Kawai E, Takayama T, Nishioka K, Motoi Y, Hattori N, Nakayama K, Yamada H, Arai H. Genetic association between RAGE polymorphisms and Alzheimer's disease and Lewy body dementias in a Japanese cohort: a case-control study. *Int J Geriatr Psychiatry.* Epub 2016 Oct 4.

Kobayashi N, Shinagawa S, Nagata T, Shimada K, Shibata N, Ohnuma T, Kasanuki K, Arai H, Yamada H, Nakayama K, Kondo K. Usefulness of DNA Methylation Levels in COASY and SPINT1 Gene Promoter Regions as Biomarkers in Diagnosis of Alzheimer's Disease and Amnestic Mild Cognitive Impairment. *PloS one.* 2016; **11:** e0168816.

Department of Bacteriology

Yoshimitsu Mizunoe, *Professor*
Tadayuki Iwase, *Assistant Professor*
Ken-ichi Okuda, *Assistant Professor*
Akiko Tajima, *Assistant Professor*
Shinya Sugimoto, *Assistant Professor*

General Summary

Research projects of our department have focused on: 1) role of gut microbe on host nitrogen cycle, 2) a novel single point mutation in domain 2 of the stress-inducible sigma factor RpoS attenuates its activity, 3) a straightforward assay for measuring glycogen levels and RpoS activity, 4) molecular mechanisms of curli biogenesis regulated by molecular chaperones, 5) roles of co-chaperones of Hsp70 in curli biogenesis, 6) staphylococcal biofilm dispersal via nuclease.

Research Activities

Role of gut microbe on host nitrogen cycle
Like oxygen, hydrogen, and carbon, nitrogen is an important element for the growth, maintenance, and survival of organisms. Nitrogen is abundantly present on earth; however, it predominantly exists in the air as molecular nitrogen, which is inactive and cannot be utilized by organisms. Compared to the amount of the bioavailable forms of other elements, the amount of bioavailable nitrogen can often be insufficient, and this insufficiency can restrict the increase in the biomass of organisms. We investigate roles of gut microbe on nitrogen cycle in host.

A novel single point mutation in domain 2 of the stress-inducible sigma factor RpoS attenuates its activity
RpoS is a sigma factor that regulates stress resistance genes in *Escherichia coli*, such as the *katE* encoding catalase HPII and the *glg* encoding glycogen synthesis proteins. Monitoring RpoS activity can provide information on the stress sensitivity of *E. coli* isolates in clinical settings because its RpoS is often mutated. In the present study, we found a novel, missense point mutation at the RpoS domain 2 in a clinical *E. coli* isolate. The mutant RpoS protein was non-functional according to the HPII activity and glycogen levels, which are positively regulated by RpoS. A reporter assay with β-galactosidase indicated that the dysfunction occurred at the transcriptional level. Substitution analysis indicated that the hydrophobicity of the amino acid at domain 2 was critical for RpoS activity. However, no RpoS activity was observed when RpoS domain 2 was substituted with the hydrophobic amino acid Pro, which can destroy the alpha-helix structure at the domain 2, suggesting that the structure near this residue may also play an important role in RpoS activity. These results contribute to a deeper understanding of RpoS regulatory mechanisms and bacterial stress responses.

A straightforward assay for measuring glycogen levels and RpoS activity
Bacterial cellular glycogen levels reflect the activity of RpoS. In this study, a straightforward assay for measuring glycogen levels and RpoS activity was developed combining the ease and simplicity of qualitative approaches. The basic principle of this assay is the iodine-glycogen reaction producing a reddish brown color that can be measured by spectrophotometer. The results indicate that the assay exhibited linearity within the range of standard solutions used (300 > μg/assay; $R^2 = 0.994$) and that the minimum detected concentration of glycogen was 10 μg/100 μl per assay. The applicability of the assay was assessed; glycogen was detected and quantified in clinical isolates with functional RpoS but not in isolates with dysfunctional RpoS; this assay constitutes a simple method for measuring RpoS activity and was successfully applied for measuring glycogen levels in human cells.

Molecular mechanisms of curli biogenesis regulated by molecular chaperones
We discovered that curli biogenesis depends on molecular chaperone DnaK, a bacterial Hsp70 homolog, by undefined mechanism(s). In this study, we showed that DnaK positively regulated expression of CsgA and CsgB, the major and minor structural components of curli. In addition, biochemical and cell biological studies demonstrated that DnaK maintained a translocation competent state of CsgA by binding to the N-terminal aggregation-prone signal sequence, leading to successful translocation of CsgA into the periplasm. These results provide mechanistic insights underlying how DnaK regulates curli biogenesis and robust biofilm formation.

Roles of co-chaperones of Hsp70 in curli biogenesis
The nucleotide-regulated cycles of the 70 kDa heat shock proteins (Hsp70s) are controlled by co-chaperones, DnaJ-domain proteins (JDPs) and nucleotides exchange factors (NEFs). The bacterial major Hsp70 system consists of DnaK, three JDPs (DnaJ, CbpA, and DjlA), and one NEF (GrpE), but co-chaperones-independent functions of DnaK remain unresolved. Here, we show the dispensability of the co-chaperones for the DnaK-regulated formation of curli, extracellular amyloid fibers involved in biofilm formation and host colonization. Full specification of the DnaK system was essential for survival at high temperature, whereas either JDPs or GrpE was dispensable for the curli production. In addition, DnaK mutants with reduced activities strongly required both co-chaperones for curli biogenesis. These results suggest that activities of the Hsp70 system can differ among individual cellular functions.

Staphylococcal biofilm dispersal via nuclease
In the staphylococcal biofilm development, bacteria formed biofilm within 8 h, however, biofilm was dispersed after 24 h. Analysis of extracellular matrix of biofilm and culture supernatant showed that this dispersal correlated with nuclease which degrades nucleic acids in the matrix. The biofilm dispersal was not detected in *nuc* mutant, which indicated that nuclease is a key factor for biofilm disassembly mechanism. Induction of biofilm dispersal by nuclease depended on pH in the culture supernatant, suggesting that environmental pH is one of the cue signals for dispersal.

Publications

Sugimoto S, Okuda K, Miyakawa R, Sato M[1], Arita-Morioka K[2], Chiba A, Yamanaka K[2], Ogura T[2], Mizunoe Y, Sato C[1] ([1]AIST, [2]Kumamoto Univ). Imaging of bacterial multicellular behaviour in biofilms in liquid by atmospheric scanning electron microscopy. *Sci Rep.* 2016; **6:** 25889.

Iwase T, Ogura Y[1], Hayashi T[1] ([1]Kyushu Univ), Mizunoe Y. Complete Genome Sequence of Klebsiella pneumoniae YH43. *Genome Announc.* 2016; **4:** pii: e00242-16.

Mizunoe Y, Sugimoto S, Okuda K. Mechanism of biofilm formation and effects of bacteriocins on MRSA biofilm. *The Journal of Farm Animal in Infectious Disease*. 2016; **5:** 113-20.

Iwase T, Ogura Y[1], Hayashi T[1], Mizunoe Y ([1]Kyushu Univ). Complete Genome Sequence of Klebsiella oxytoca Strain JKo3. *Genome Announc.* 2016; **4:** pii: e01221-16.

Department of Tropical Medicine

Hirotaka Kanuka, *Professor* Kenji Ishiwata, *Associate Professor*

General Summary

There is a great need to develop novel parasite control strategies because of the failures of current eradication approaches and the logistical difficulties to implement them. One interesting aspect of these diseases is that the vector arthropods that transmit the pathogens can mount immune responses against the infection that will kill a large proportion of parasites. Our group is pursuing research that covers 4 topics: (1) vector–parasite interactions, (2) infection response in intermediate host, (3) immune responses to helminth infection, and (4) vector epidemiology.

Research Activities

Intestinal mucus barrier against re-infection of a gastrointestinal nematode
Heligmosomoides polygyrus (Hp), one of the murine gastrointestinal nematodes, infection has been employed as a re-infection model of parasite immunology. The swallowed larvae of infective stage once penetrate mucosa into muscularis where they grow up, then appear back to the lumen of the small intestine in mice. Although Hp persist to reside on the mucosa for over two months in initial infection, re-infected Hp after deworming is expelled from the small intestine soon after establishment on the mucosa. Many immunological studies focused on the protective immune response against Hp in the lumen of the gut, however, the number of re-infected Hp just after establishment on the mucosa is lower than those of initially infected Hp suggesting that acquired protective immune response to Hp also includes penetration blockade of the larvae at the mucosa surface. Here we confirmed the blockade and examined related mechanisms. Infective larvae were inoculated 4 weeks after deworming of initial infection, then the growing larvae in the muscularis of the small intestine were counted under the dissecting microscope on day 6 after the challenge infection. Number of larvae in the immune mice was lower than that of naïve mice. The statistical significant difference in the total numbers of larvae was derived from the difference in upper part 1/6 of the small intestine where initially infected larvae prefer to penetrate and grow up. The blockade persisted for 10 weeks after deworming, and depended on Th2 cells and Fcε receptor. Results suggest that acquired protective immunity against Hp consists of two mechanisms; 1) accelerated expulsion of adult worms from the gut lumen, and 2) blockade of larval penetration into gut mucosa. The latter newly found-barrier mechanism would be valuable for the mucosal vaccine strategy.

Toward the establishment of more suitable strain of Lucilia sericata for maggot debridement therapy
Maggot debridement therapy (MDT) is one of biotherapy that involves the application of

green bottle fly, *Lucilia sericata*, larvae (Maggot) to the necrotic wounds. Maggot application to the wound promotes removal of necrotic tissues, disinfection, and healing. Toward the establishment of more suitable strain of fly for MDT, a measure to evaluate the ability of maggot for debridement has been developed. Maggots fed on nonliving human tissue, removed during surgical debridement, or mixture of ground beef and pork were compared for their growth by weighing. Each maggots group showed respective rise curves, indicating maggot actually eats nonliving human tissues. Our result suggests the necessity of evaluating maggot for their debridement ability prior to the application to MDT. Controlling maggot pupation by reducing acdysteroids was also examined, which could lead to the extension of days between each treatment. Maggots injected with Ecdysteroid-22-oxidase (E22O), an ecdysteroid-inactivating enzyme, showed extended larval period without onset of pupation, thus suggesting potency of improved medical maggots applicable on wound for longer period. Our evaluation and improvement of medical maggots potentially amend therapeutic effect and patient outcome with MDT.

Elucidation of molecular basis of tick host detection

Tick-borne diseases present major public health issues worldwide. Blood-sucking insects dedicate many of their sensory abilities to detect and follow the physical and chemical signals emitted by their hosts. In general, it is known that mosquitoes are remarkable for their ability to locate blood meal using host body emanations such as CO_2, smell, and heat acting as strong mosquito attractants. Recently, evidence for thermosensitive sensilla on mosquito appendages has been uncovered. It was reported that the activation of a transient receptor potential, via ion channels involved in various types of sensory reception, including thermo-, chemo-, mechano-, and photoreception, is caused by an increase in temperature from 25°C to 37°C in mosquitoes. On the contrary, tick forelegs are known as antennae necessary for the recognition of distant hosts using the Haller's organ, a sensory structure containing sensilla on the dorsal surface of the leg. To understand the molecular processes by which ticks sense external thermal signals, we investigated the impact of ambient temperature on larval, nymph, and adult stage locomotion. To verify a previously unknown role for the tick Haller's organ during host recognition, we used an automated device that was able to quantify selected host-detecting behaviors (Etho Vision XT, Sophia Scientific). *Haemaphysalis longicornis* strongly reacted to a stimulus of carbon dioxide, but *Amblyomma testedarium* did not react. This data suggests that response behaviors to inducing stimuli, such as heat or carbon dioxide, are different depending on the host. We will investigate the participation of TRPA1 molecules in host exploratory behavior by the behavior analysis system.

Dissection of blood sucking behavior of mosquitoes

Exploring the molecular mechanism of blood sucking behavior of female mosquitoes is one of the critical steps to fight against vector-borne disease such as dengue and malaria, since pathogens are transmitted when mosquitoes are gorging on blood. It has been known that ATP in blood serves as a phagostimulant. To identify the candidate chemoreceptor that perceives ATP in mosquitoes, we focused on gustatory receptors (Grs) of *Drosophila melanogaster* from following two reasons: 1) sequence of P2X receptor that per-

ceives ATP in broad animals is missing in mosquitoes and *Drosophila*, and 2) nonvolatile compounds are generally perceived by Grs in *Drosophila*. Two-choice behavior assay showed *Drosophila* also show feeding preference to ATP and not to adenosine. Each 116 RNAi strain against 53 Grs was specifically expressed in neurons and feeding preference was examined by two-choice behavior assay. Among 53 Grs, 17 Grs seem to contribute to perceive ATP and 7 Grs to adenosine. Some of these Grs are conserved in mosquitoes, suggesting that they can be the strong candidates of ATP/adenosine receptors in mosquitoes. Thus, we revealed that *Drosophila* shows the same feeding preference to ATP as mosquitoes, and Grs seem to intermediate this chemoreception.

Seroepidemiology and risk assessment of Toxoplasma gondii *infection in HIV/AIDS patients*

In HIV-infected patients, AIDS develops with decreased CD4 positive lymphocytes. Toxoplasma encephalitis is one of the AIDS indicator diseases that its risk increases when CD4 positive lymphocyte becomes 100/μl or less. Majority of cases are caused by reactivation of bradizoites in brains, which forms latent infections. However, there is no adequate assessment of toxoplasma seroprevalences and its risk factors among Japanese HIV-infected patients. We collected serums from 400 HIV-infected patients who visited our hospital outpatient clinic and conducted serological evaluation of *T. gondii* specific-antibody levels by anti-toxoplasma IgG (ELISA), with confirmation by Sabin-Feldman Dye Test. As a result, 33 cases (8.3%) of patients were *T. gondii* IgG antibody positive, and every case was Sabin-Feldman Dye test positive. The obtained prevalence of seropositivity was equivalent to the previous survey that was conducted in pregnant women in Japan; there was no correlation with HIV infection. Also, a correlation between seropositivity and with a history of cat rearing was found, rather than having a habit of consuming rare meat from the questionnaire survey to the participants. Consumption of rare bivalve, which is reported to be a probable risk factor in other studies, was not correlated with toxoplasma infection in our study.

Publications

Teshima T*[1]*, Onoe H*[1]*, Tottori S*[1]*, Aonuma H, Mizutani T*[1]*, Kamiya K*[1]*, Ishihara H*[1]*, Kanuka H, Takeuchi S*[1] *(*[1]*Univ Tokyo). High-Resolution Vertical Observation of Intracellular Structure Using Magnetically Responsive Microplates. *Small.* 2016; **212:** 3366-73.

Sakuma C, Satio Y*[1]*, Umehara T*[1]*, Kamimura K*[2]*, Maeda N*[2]*, Mosca TJ*[3]*, Miura M*[1]*, Chihara T*[4] *(*[1]*Univ Tokyo,* [2]*Tokyo Met Inst Med Sci,* [3]*Stanford Univ,* [4]*Hiroshima Univ). The Strip-Hippo pathway regulates synaptic terminal formation by modulating actin organization at the *Drosophila* neuromuscular synapses. *Cell Rep.* 2016; **16:** 1-9.

Kiniwa T*[1]*, Enomoto Y*[1]*, Terazawa N*[1]*, Omi A*[1]*, Miyata N*[1]*, Ishiwata K, Miyajima A*[1] *(*[1]*Univ Tokyo). NK cells activated by interleukin-4 in cooperation with interleukin-15 exhibit distinctive characteristics. *Proc Natl Acad Sci USA.* 2016; **113:** 10139-744.

Chinuki Y*[1]*, Ishiwata K, Yamaji K, Takahashi H*[1]*, Morita E*[1] *(*[1]*Shimane Univ). Haemaphysalis longicornis tick bites are a possible cause of red meat allergy in Japan. *Allergy.* 2016; **71:** 421-5.

Department of Public Health and Environmental Medicine

Hiroyuki Yanagisawa, *Professor*
Shingo Yogosawa, *Assistant Professor*
Machi Suka, *Associate Professor*
Wataru Yoshioka, *Assistant Professor*

General Summary

Our major research projects in the 2016 academic year focused on: (1) effects of nanomaterials on chromosomal abnormality; (2) effects of Zn-deficiency on the expression of interleukins associated with a decrease in anti-inflammatory M2 macrophages; (3) mechanisms of a developmental-stage specific toxicity of lithium carbonate; (4) molecular approaches toward cancer chemoprevention with food factors; (5) effects of arsenic on the cholesterol metabolism; (6) the decompression stress in the hyperbaric work; (7) menopause-specific health literacy; (8) help-seeking intentions for mental illness; (9) impact of postprandial hyperglycemia on the incidence of cardiovascular events and all-cause mortality in type 2 diabetes patients; and (10) effects of polaprezinc, a carnosine-zinc complex, on pica and polydipsia or binge eating.

Research Activities

Experimental Medicine

1. Effects of nanomaterials on chromosomal abnormality in CHL/IU cells

We examined induction of micronuclei by exposure of AlO_2 or CeO_2 nanoparticles in both metabolic activation and inactivation on chinese hamster CHL/IU cells.

2. Mechanisms responsible for a decrease in anti-inflammatory M2 macrophages in the spleen and role of IL-4 in Zn-deficient rats

Zn deficiency causes growth retardation and the dysfunction of immune and reproductive systems. We found that IL-4 and IL-13 mRNA expression was significantly lower in the Zn deficient (ZnD) group compared with that in the standard diet (SD) group. The number of IL-4 and Il-13 positive lymphocytes in the spleen was reduced in the ZnD group compared to the SD group.

3. Kidney damage induced by an overproduction of PGE2

Aberrant upregulation of PGE2 synthesis results in polyuria and hydronephrosis, which was demonstrated by us using dioxin-exposed mouse model. In this year, we found that lithium carbonate, which upregulates PGE2 synthesis system, induced polyuria and hydronephrosis in mouse neonates. Our findings suggest that those chemicals upregulating PGE2 synthesis system are hazardous to the mammalian kidney in the developing stage.

4. Molecular approaches toward cancer chemoprevention with food factors

We have attempted to establish an evidence-based cancer prevention method using food factors. We elucidated that equol, an isoflavandiol metabolized from daidzein, a type of isoflavone, from bacterial flora in the intestines, enhanced the inhibitory effect of brassinin, a phytoalexin from *Brassica* vegetables, on the growth of cancer cells via cell-cycle

arrest at G1 phase with up-regulation of CDK inhibitors (p21 and p27) and induced caspase-dependent apoptosis accompanied with loss of mitochondrial membrane potential.
5. The effect of arsenic on the cholesterol metabolism
Recent epidemiological studies suggest that arsenic exposure involved in atherosclerosis. In this study, we focused on the effect of arsenic in the cholesterol metabolism by using Hepa1c1c7 cells. Gene expression analysis showed that arsenic suppresses the expression of Abca1 transporter which involved in HDL efflux. To elucidate the mechanisms of inhibition of Abca1, we are now focusing on LXR pathway.
6. A study of the decompression stress in the hyperbaric work
Exposure to a hyperbaric environment and the subsequent decompression to the surface may cause the decompression stress. The level of decompression stress would be related to the risk of decompression sickness (DCS). However, there are no biomarkers for DCS. Bubbles in the body after decompression and the number of HHV-6 in saliva may permit use of such biomarkers for the decompression stress.

Epidemiology, evidence-based medicine, investigation, and medical informatics
1. Menopause-specific health literacy
A web-based survey was conducted among Japanese women aged 30-59 years to assess menopause-specific health literacy. Participants were presented with a vignette describing a typical case of menopausal symptoms and were then asked a series of questions to assess their recognition of the disorder and intention, attitude, subjective norm, perceived behavioral control, and available information related to seeking medical care.
2. Help-seeking intentions for mental illness
A web-based survey was conducted among Japanese adults aged 20-59 years to explore possible differences in help-seeking intentions for early signs of mental illness. Psychological problems (insomnia and depression) were significantly less likely to induce help-seeking intentions than physical problems (headache and vertigo).
3. Impact of postprandial hyperglycemia at clinic visits on the incidence of cardiovascular events and all-cause mortality in patients with type 2 diabetes
Postprandial hyperglycemia represented by the mean level of 2-hour post-breakfast blood glucose at clinic visits is associated with the incidence of cardiovascular disease and all-cause mortality independently of the mean HbA1c level in type 2 diabetes patients.
4. Effects of polaprezinc on pica and polydipsia
We performed an open-label trial to evaluate the effects of polaprezinc in pica patients with/without polydipsia and to also examine the changes in serum concentrations of brain-derived neurotrophic factor (BDNF) before and after polaprezinc treatment.
5. Effects of polaprezinc on binge eating
We performed an open-label trial to evaluate the effects of polaprezinc on binge eating in patients with bulimia nervosa or binge-eating disorder. We also assessed the EDE-Q (Eating Disorder Examination Questionnaire), the QIDS-SR16 (Quick Inventory of Depressive Symptomatology-Self-report), body weight, blood biochemistry, and plasma concentrations of BDNF over the course of trial.

Publications

Yanagisawa H, Kawashima T, Miyazawa M, Ohshiro T. Validity of the copper/zinc ratio as a diagnostic marker for taste disorders associated with zinc deficiency. *J Trace Elem Med Biol.* 2016; **36:** 80–3.

Suka M, Taniuchi A[1], Igarashi S[1], Yanagisawa H, Ishizuka B[1] (1St Marianna Univ Sch Med). Menopause-specific health literacy in Japanese women. *Maturitas.* 2016; **91:** 51–9.

Yoshioka W, Kawaguchi T[1], Nishimura N[1], Akagi T[1], Fujisawa N[1], Yanagisawa H, Matsumura F[2], Tohyama C[1] ([1]Univ Tokyo, [2]Univ California, Davis). Polyuria-associated hydronephrosis induced by xenobiotic chemical exposure in mice. *Am J Physiol Renal Physiol.* 2016; **311:** 752–62.

Takakura K, Ito Z, Suka M, Kanai T, Matsumoto Y, Odahara S, Matsudaira H, Haruki K, Fujiwara Y, Saito R, Gocho T, Nakashiro K[1], Hamakawa H[1], Okamoto M[2], Kajihara M, Misawa T, Ohkusa T, Koido S ([1]Ehime Univ, [2]Kitasato Univ). Comprehensive assessment of the prognosis of pancreatic cancer: Peripheral blood neutrophil-lymphocyte ratio and immunohistochemical analyses of the tumor site. *Scand J Gastroenterol.* 2016; **51:** 610–7.

NCD Risk Factor Collaboration. A century of trends in adult human height. *Elife.* 2016; **5**.

NCD Risk Factor Collaboration. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *Lancet.* 2016; **387:** 1377–96.

NCD Risk Factor Collaboration. Worldwide trends in diabetes since 1980: pooled analysis of 751 population-based measurement studies with over 4.4 million participants. *Lancet.* 2016; **387:** 1513–30.

NCD Risk Factor Collaboration. Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19.1 million participants. *Lancet.* 2017; **389:** 37–55.

Suka M, Yamauchi T[1], Sugimori H[2] ([1]National Center of Neurology and Psychiatry, [2]Daito Bunka Univ). Help-seeking intentions for early signs of mental illness and their associated factors: comparison across four kinds of health problems. *BMC Public Health.* 2016; **16:** 301.

Usui J[1], Tawara-Iida T[1], Takada K[2], Ebihara I[3], Ueda A[4], Iwabuchi A[5], Ishizu T[6], Iitsuka T[7], Takemura K[8], Kawamura T[1], Kaneko S[1], Sakai K[7], Kai H[1], Gomibuchi T[9], Nagata M[1], Kobayashi M[10], Koyama A[1], Suka M, Radhakrishnan J[11], Yamagata K ([1]Univ Tsukuba, [2]Tsukuba Gakuen Hospital, [3]Mito Saiseikai General Hospital, [4]Hitachi General Hospital, Hitachi, [5]Kensei General Hospital, [6]Tsukuba Central Hospital, [7]Ibaraki Seinan Medical Center, [8]Takemura Nephrology Clinic, [9]Univ Tokyo, [10]Tokyo Medical Univ Ibaraki Medical Center, [11]Columbia Univ Medical Center). Temporal changes in post-infectious glomerulonephritis in Japan (1976–2009). *PLoS One.* 2016; **11:** e0157356.

Kido T, Sugaya C[1], Yanagisawa H, Sugita-Konishi Y[2] ([2]Azabu Univ), Itai K[3] ([3]Iwate Univ), Tsunoda M[1] ([1]Kitasato Univ). The subacute administration of fluoride in drinking water increased serum blood urea nitrogen and but not creatinine clearance in ICR-derived glomerulonephritis mice. *Fluoride.* 2016; in press.

Akiyama M[1], Sowa Y[1], Taniguchi T[1], Watanabe M[1], Yogosawa S, Kitawaki J[1], Sakai T[1] ([1]Kyoto Pref Univ Med). Three-Combined Treatment, a Novel HDAC Inhibitor OBP-801/YM753, 5-Fluorouracil and Paclitaxel, Induces G2-Phase Arrest Through the p38 Pathway in Human Ovarian Cancer Cells. *Oncol Res.* 2017 Sep 21; **25:** 1245–1252. Epub 2017 Jan 23.

Takao T[1], Suka M, Yanagisawa H, Iwamoto Y[1] ([1]Institute for Adult Diseases Asahi Life Foundation). Impact of postprandial hyperglycemia at clinic visits on the incidence of cardiovascular events and all-cause mortality in patients with type 2 diabetes. *J Diabetes Investig.* 2017 Jul; **8:** 600–8. Epub 2017 Jan 24.

Department of Forensic Medicine

Kimiharu Iwadate, *Professor*
Kyoko Maebashi, *Assistant Professor*
Kenji Fukui, *Assistant Professor*

General Summary

Our main research projects in 2016 have mainly focused on forensic pathology, DNA analysis, and forensic toxicology as has happened in the past. Much of the research was based on forensic practice. The details of our research are described below.

Research Activities

Forensic Pathology

1. Values of Acrolein and several markers, of which patients died in the bath tabs

In Japan, many people die in the bath tabs, and it is said that transient ischemic attack (TIA) is contributory to death there. We determined the values of Protein conjugated acrolein (PC-Acro), polyamine oxidase (SMO, AcPAO etc.) and several markers, of 10 cases died in the bath tabs and 10 control cases, in our forensic autopsy. In the results of analysis, we are not able to get significantly different between groups. It might be because TIA doesn't play a part in death in the bath tabs, number of cases are too low, and the value fluctuate due to postmortem change. Therefore we need to increase number of cases and study the intergradation of each value due to the time since death.

DNA analysis

1. Identification of war-dead remains with DNA analysis

We performed identification of war-dead remains that recovered and repatriated from the former Soviet Union and southern area by means of DNA analysis as part of the war-dead remains return project of the Ministry of Health, Labor and Welfare. For genetic markers we used single nucleotide polymorphisms of hypervariable region of mitochondrial DNA and short tandem repeats of nuclear DNA.

2. The detection and analysis of X chromosome Short tandem repeats (X-STR) locus

The analysis of STRs located on the X chromosome is known to be useful in kinship testing.

We performed detection and population genetic study of a novel tetranucleotide X-STR locus in the present study. We analyzed sequence structure of novel X-STR, appearance frequency of Alleles and forensic statistics data. And we registered these data with the International Nucleotide Sequence Databases (ISDN). We are going to investigate relevance with other X-STR by linkage analysis.

Forensic toxicology

1. Medicines and poisonous substances (abuse drugs, alcohol, carbon monoxide, cyanide, and agricultural chemicals) suspected to have caused deaths were quantitatively analyzed

with gas chromatography, gas chromatography/mass spectrometry, liquid chromatography-tandem mass spectrometry, and spectrum photometry in tissue specimens obtained at autopsy.
In addition, for the purpose of quality control of drug analysis, we conducted a blind test twice a year in collaboration with other universities.
2. For 158 autopsy cases, drug screening analysis was performed. As a result, there were three cases in which caffeine corresponding to a lethal area was detected. In addition, there were 14 cases where caffeine above the poisoning region was detected. Though one of them was drug addiction due to multiple drug administration, evidence of ingestion of drugs, caffeine tablets and a large amount of energy drinks was not observed in the other 13 cases. From the situation and the environment of the deceased, it is presumed that it is due to intake of luxury goods on a daily basis, and none caffeine directly linked to the cause of death.
3. Trace analysis of the odor in the dissected room was performed using Two-Dimensional Gas Chromatography with Time-of-Flight Mass Spectrometer.

Radiocarbon analysis
1. Establishment of date of birth
We studied the estimation of date of birth from carbon-14 isolated from a tooth. To apply this method to the forensic practice, we have examined the amount of minimum enamel and dentin required for the analysis.

Publications

Nishi T, Nakamura T, Honda K. Detection of a novel X-chromosomal short tandem repeat marker in Xq28 in four ethnic groups. *Leg Med (Tokyo).* 2016; **19:** 43-6.
Irii T, Maebashi K, Fukui K, Sohma R, Matsumoto S, Takasu S, Iwadate K. Development of a dual test procedure for DNA typing and methamphetamine detection using a trace amount of stimulant-containing blood. *Leg Med (Tokyo).* 2016; **20:** 53-60.

Department of Internal Medicine
Division of Gastroenterology and Hepatology

Masayuki Saruta, *Chairman and Professor*
Shigeo Koido, *Associate Professor*
Tomohisa Ishikawa, *Associate Professor*
Kazuhiko Koike, *Assistant Professor*
Shinichiro Uetake, *Assistant Professor*
Akiyoshi Kinoshita, *Assistant Professor*
Toshifumi Okusa, *Professor*
Atushi Hokari, *Associate Professor*
Mika Matsuoka, *Assistant Professor*
Seiji Arihiro, *Assistant Professor*
Kan Uchiyama, *Assistant Professor*
Makoto Mitsunaga, *Assistant Professor*

Research Activities

Alimentary Tract

1. Examination of new biomarkers to assess disease activity in inflammatory bowel disease

1) Prostaglandin E-Major Urinary Metabolite as a Reliable Surrogate Marker for Mucosal Inflammation in Ulcerative Colitis

We evaluated whether prostaglandin E-major urinary metabolite (PGE-MUM) can be used as a biomarker for ulcerative colitis (UC). Areas under the receiver operating characteristic curves of simple clinical colitis activity index, Mayo endoscopic scoring, and Matts' grading (Histologic Activity) for PEG-MUM were each higher than for CRP.

The main advantage of PGE-MUM appears to be differentiation of colonoscopic or histologic remission from active disease in UC. On the other hand, this maker of UC patients in remission was lower compared to healthy volunteers. By comparison to CRP level, PGE-MUM level demonstrated better sensitivity for reflecting UC activity, especially in cases of histologic inflammation, and thus seems to be a better evaluator of mucosal healing.

According to this result, we have conducted a comparison trial to detect a most reliable marker for detecting endoscopic mucosal healing in UC patients among immunochemical fecal occult blood test, fecal calprotectin, and PGE-MUM.

2) The clinical benefit of procalcitonin to assess disease activity and severity in inflammatory bowel disease

Levels of procalcitonin (PCT) are relevant to immunologic responses that contribute to systemic inflammation responses and septic shock. PCT demonstrated activity of chronic inflammatory and autoimmune diseases like Wegener's granulomatosis. Herein, we hypothesed the serum PCT level might be helpful to predict the disease activity of inflammatory bowel disease (IBD); Crohn's disease (CD), ulcerative colitis (UC) or intestinal Behcet's disease (Int/BD). The Serum PCT levels were correlated to activity of CD, Int/BD, not UC. These levels were helpful to distinguish severe active to fulminant CD from mild to moderate active CD, and may serve as a new serological marker of disease activity as well as CRP.

2. The development of treatment using phototherapeutic effect based on fluorescence molecular imaging

We have developed a method for boosting HER2-specific cancer theranostics utilizing

near-infrared light and HER2-specific monoclonal antibody-photoabsorber conjugates.
3. Nutritional treatment for inflammatory bowel disease
The intake of n-3 PUFA and the subsequent associated efficacy for the maintenance of remission may be achieved by understanding the importance of n-3 diet therapy.
4. A lymph node metastatic risk factor of the esophageal superficial carcinoma
Performing statistical analysis about a lymph node metastatic risk factor of the esophageal superficial carcinoma, a vascular invasion evaluation using the special staining procedure was the strongest risk factor.

Liver
1. The development of targeting therapy for cancer stem cells in liver cancers
The only curative treatments for primary liver cancers are surgical resection for early-stage patients. However, most patients are diagnosed at advanced stages by which time extant therapies are ineffective. Therefore, the identification of novel molecules that can become targets for future therapies is urgently needed. We have reported that 1) SALL4 regulates cell fate decision in hepatic stem/progenitor cells during normal liver development 2) SALL4 is indicative of aggressiveness and poor prognosis and maintains the stemness of cancer stem cells in liver cancers. Further analyses on cancer stem cell-mediated mechanisms may provide a novel future therapeutic strategy against liver cancers.
2. Pathogenesis, mRNA and miRNA expression profiling of primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH)
The pathogenesis is unknown in autoimmune liver disease. To investigate the pathogenesis and identify novel therapeutic targets, we analyzed mRNA and miRNA expression in CD4+ T cells derived from 14 PBC patients using microarray analyses. We found that decreased expression of four miRNAs (miR-425, -181a, -181b, -374b) which dysregulate TCR signaling in PBC-CD4 T+ cells. Especially, the decreased miR-425 expression strongly induced inflammatory cytokines via N-Ras upregulation in the TCR signaling pathway, suggesting that the restoration of decreased mir-425 or the suppression of N-Ras may be a promising immunotherapeutic strategy against PBC.
3. The relationship between nutritional condition and neuropsychological test results in liver cirrhosis patients
Liver cirrhosis (LC) cases complicate minimum hepatic encephalopathy (MHE) and have caused some traffic accidents and communication problems. Neuropsychological disturbance is typical of MHE. However, the diagnostic criteria for MHE have not yet been clarified. We studied the pathophysiological findings of MHE using a neuropsychological test (NPT) and food frequency questionnaire (FFQg). 17% of LC patients were Digit Symbol Test (DST)-abnormal. DST-abnormal was related to Child-Pugh score, serum albumin, Branched chain amino acid & Tyrosine Ratio (BTR), nutrient intake as usual energy intake, and fat energy ratio. Thus, NS using the FFQg may be a useful method to prevent MHE.
4. The investigation of Frailty index in elderly digestive disease patients
We evaluate whether simplified Frailty Scores are associated with clinical outcomes or adverse outcomes after treatments in elderly patients over 80 years old with digestive disease.

Gall bladder andPancreas

1. The investigation of Wilm's tumor protein 1 (WT1)-pulsed dendritic cell vaccines for the advanced pancreatic cancer patients

Prolonged low levels of plasma IL-6/-8 in pancreatic ductal adenocarcinoma (PDA) patients may be a prognostic marker for the clinical outcomes of chemoimmunotherapy.

2. The trend of disease of the hepatobiliary system in super-aging society

There has been a dramatic increase in the average life expectancy in Japan. Therefore, the opportunities to examine super-elderly patients over 80 years old with digestive disease have significantly increased in our hospital. Thus, we investigate the clinical characteristics and outcomes of super-elderly patients over 80 years old with digestive disease, including hepatocellular carcinoma, pancreatic cancer, chronic hepatitis C and acute cholecystitis.

3. The investigation of the relationship between biliary tract disease and inflammation-based prognostic scores

Inflammation-based prognostic scores have been reported to have prognostic value in patients with various types of cancer. These inflammation-based prognostic scores have also been shown to correlate with outcomes or disease severity in patients with, sepsis, acute heart failure, and Crohn's disease. Therefore, we evaluate whether the inflammation-based prognostic scores are associated with disease severity in patients with acute cholecystitis or acute cholangitis.

Publications

Torisu Y, Nakano M, Takano K, Nakagawa R, Saeki C, Hokari A, Ishikawa T, Saruta M, Zeniya M. Clinical usefulness of ursodeoxycholic acid for Japanese patients with autoimmune hepatitis. *World J Hepatol.* 2017; **9:** 57-63.

Ueda K, Kinoshita A, Koike K, Nishino H. Clinical outcomes of super-elderly pancreatic cancer patients who are not considered to be suitable for surgical resection. *Jikeikai Medical Journal.* 2016; **63:** 55-61.

Horikiri T, Hara H, Saito N, Araya J, Takasaka N, Utsumi H, Yanagisawa H, Hashimoto M, Yoshii Y, Wakui H, Minagawa S, Ishikawa T, Shimizu K, Numata T, Arihiro S, Kaneko Y, Nakayama K, Matsuura T, Matsuura M, Fujiwara M (Japanese Red Cross Medical Center), Okayasu I (Kitasato University School of Medicine), Ito S (IDAC Theranostics, Inc), Kuwano K. Increased levels of prostaglandin E-major urinary metabolite (PGE-MUM) in chronic fibrosing interstitial pneumonia. *Respir Med.* 2017 Jan; **122:** 43-50. Epub 2016 Nov 24.

Akasaki Y, Kikuchi T, Homma S, Koido S, Ohkusa T, Tasaki T, Hayashi K, Komita H, Watanabe N, Suzuki Y, Yamamoto Y, Mori R, Arai T, Tanaka T, Joki T, Yanagisawa T, Murayama Y. Phase I/II trial of combination of temozolomide chemotherapy and immunotherapy with fusions of dendritic and glioma cells in patients with glioblastoma. *Cancer Immunol Immunother.* 2016 Dec; **65:** 1499-509.

Hidaka A, Sasazuki S, Matsuo K, Ito H, Charvat H, Sawada N, Shimazu T, Yamaji T, Iwasaki M, Inoue M, Tsugane S. CYP1A1, GSTM1, and GSTT1 genetic polymorphisms and gastric cancer risk among Japanese: A nested case-control study within a large-scale population-based prospective study. *Int J Cancer.* 2016; **139:** 759-68.

Svensson T, Yamaji T, Budhathoki S, Hidaka A, Iwasaki M, Sawada N, Inoue M, Sasazuki S, Shimazu T, Tsugane S. Alcohol consumption, genetic variants in the alcohol- and folate metabolic pathways and colorectal cancer risk: the JPHC Study. *Sci Rep.* 2016 Nov 9; **6:** 36607.

Ogawa T, Sawada N, Iwasaki M, Budhathoki S, Hidaka A, Yamaji T, Shimazu T, Sasazuki S, Narita Y, Tsugane S. Coffee and green tea consumption in relation to brain tumor risk in a Japanese population. *Int J Cancer.* 2016; **139:** 2714-21.

Goto A, Noda M, Sawada N, Kato M, Hidaka A, Mizoue T, Shimazu T, Yamaji T, Iwasaki M, Sasazuki S, Inoue M, Kadowaki T, Tsugane S. High hemoglobin A1c levels within the non-diabetic range are associated with the risk of all cancers. *Int J Cancer.* 2016; **138:** 1741-53.

Shibahara-Sone, Gomi A, Iino T, Kano M, Nonaka C, Watanabe O, Miyazaki K (Yakult Central Institute), Ohkusa T. Living cells of pro-

biotic Bifidobacterium bifidum YIT 10347 detected on gastric mucosa in humans. *Benef Microbes.* 2016 Jun; **7:** 319–26.

Reviews and Books

Oikawa T. Cancer Stem Cells and Their Origin of Primary Liver and Biliary Tract Cancers. *Hepatology.* 2016; **64:** 645–51.

Aizawa Y, Abe H, Sugita T, Seki N, Chuganji Y, Furumoto Y, Sakata A. Centrilobular zonal necrosis as a hallmark of a distinctive subtype of autoimmune hepatitis. *Eur J Gastroenterol Hepatol.* 2016 Apr; **28:** 391–7.

Kinoshita A, Koike K, Nishino H. Clinical features and prognosis of elderly patients with hepatocellular carcinoma not indicated for surgical resection. *Geriatr Gerontol Int.* 2017; **17:** 189–201.

Koido S, Okamoto M (Kitasato Univ), Shimodaira S (Shinshu Univ), Sugiyama H (Osaka Univ). Wilms' tumor 1 (WT1)–targeted cancer vaccines to extend survival for patients with pancreatic cancer. *Immunotherapy.* 2016 Nov; **8:** 1309–20.

Koido S. Dendritic–Tumor Fusion Cell–Based Cancer Vaccines. *Int J Mol Sci.* 2016 May 26; **17:** pii: E828.

Kajihara M, Takakura K, Kanai T, Ito Z, Matsumoto Y, Shimodaira S[1], Okamoto M[2], Ohkusa T, Koido S ([1]Shinshu Univ, [2]Kitasato Univ). Advances in inducing adaptive immunity using cell–based cancer vaccines: Clinical applications in pancreatic cancer. *World J Gastroenterol.* 2016 May 14; **22:** 4446–58.

Kajihara M, Takakura K, Kanai T, Ito Z, Saito K, Takami S, Shimodaira S[1], Okamoto M[2], Ohkusa T, Koido S ([1]Shinshu Univ, [2]Kitasato Univ). Dendritic cell–based cancer immunotherapy for colorectal cancer. *World J Gastroenterol.* 2016 May 7; **22:** 4275–86.

Department of Internal Medicine
Division of Neurology

Yasuyuki Iguchi, *Professor*
Masahiko Suzuki, *Associate Professor*
Chizuko Toyoda, *Associate Professor*
Takashi Hasegawa, *Assistant Professor*
Renpei Sengoku, *Assistant Professor*
Shusaku Omoto, *Assistant Professor*
Yuka Terasawa, *Assistant Professor*
Hisayoshi Oka, *Professor*
Hiroshi Yaguchi, *Associate Professor*
Kazutaka Matsui, *Assistant Professor*
Yu Kono, *Assistant Professor*
Hidetaka Mitsumura, *Assistant Professor*
Toshiaki Hirai, *Assistant Professor*

General Summary

Our researches in 2016 consist of the following areas: 1) cerebrovascular disease 2) neurodegenerative disease, and 3) autoimmune disease. We did not only clinical researches but started to do basic researches regarding these areas.

Research Activities

Cerebrovascular disease
We started a new multi-center study about the clinical characteristics in juvenile stroke and continued to take part in other multi-center studies (study about wake-up stroke, study about emobilc stroke undetermined sources and study about stroke under anticoagulation therapy). Secondary, we established a pioneer project in Japan, consisting of stroke coordinate nurses (SCNs) who support the hyper-acute treatment including thrombolysis. Then, we did several prospective and retrospective studies from our stroke care unit (SCU) registry. The main thema of our clinical studies are follows: 1) right-to-left shunt (RLS) evaluation using a novel probe (pasteable soft ultrasound probe; PSUP), 2) Cerebral microbleeds in cerebrovascular disease, 3) Nonstenotic carotid plaque of ipsilateral embolic stroke of undetermined source, 4) the factors associated with hematoma expansion (HE) in acute intracerebral hemorrhage patients. On the other hand, we started the basic research to establish animal model (especially primate model) of cerebral infarction supported by The Jikei University Research Fund and Grant-in-Aid for Young Scientists (B).

Neurodegenerative disease
1. Parkinson's disease (PD) and the related disorders
We studied the cardiovascular autonomic dysfunction in patients with PD and the related disorders and we reported that The patients with reduced nocturnal blood pressure fall had a low cardiac uptake in123I-MIBG scintigraphy. We also evaluated the influence of dopamine agonist for nocturnal blood pressure fall obtained from 24-hour ambulatory blood pressure monitoring test Nocturnal blood pressure fall may improve in patients which some dopamine agonist had administered. Moreover, we clarified olfactory dysfunction and digestive dysfunction are dissociated in de novo PD. We also wrote the

review discussing methodological problems inherent in wearable devices such as accelerometers and gyroscopes that quantify motor abnormalities, including decreased activity, gait disturbances, freezing of gait, falling, tremor and dyskinesia, as well as non-motor signs, such as sleep disturbances and autonomic dysfunctions in PD.

2. Swallowing function and respiratory function in the patients with neurodegenerative disease

We clarified that percutaneous endoscopic gastrostomy (PEG) with noninvasive positive pressure ventilation seemed to be a valid method for dysphagic ALS patients with respiratory failure.

Autoimmune disease

We continued to study human papillomavirus (HPV) vaccination associated with neuroimmunopathic syndrome (HANS). We demonstrated relative hypo-perfusion area in 3D-SSP with SEE analysis was most prominent in cingulate gyrus in patients after HPV vaccinations. Our study strongly suggested that various clinical symptoms with the HANS patients were caused by central nervous system impairment after HPV vaccination.

Publications

Mitsumura H, Miyagawa S, Komatsu T, Hirai T, Kono Y, Iguchi Y. Relationship between Vertebral Artery Hypoplasia and Posterior Circulation Ischemia. *J Stroke Cerebrovasc Dis.* 2016; **25:** 266-9.

Omoto S, Hasegawa Y, Sakai K, Matsuno H, Arai A, Terasawa Y, Mitsumura H, Iguchi Y. Common Carotid Artery Stump Syndrome Due to Mobile Thrombus Detected by Carotid Duplex Ultrasonography. *J Stroke Cerebrovasc Dis.* 2016; **25:** 23: e205-7.

Umehara T, Nakahara A, Matsuno H, Toyoda C, Oka H. Body weight and dysautonomia in early Parkinson's disease. *Acta Neurol Scand.* 2017; **135:** 560-7.

Umehara T, Nakahara A, Matsuno H, Toyoda C, Oka H. Predictors of postprandial hypotension in elderly patients with de novo Parkinson's disease. *J Neural Transm (Vienna).* 2016; **123:** 1331-9.

Umehara T, Matsuno H, Toyoda C, Oka H. Clinical characteristics of supine hypertension in de novo Parkinson's disease. *Clin Auton Res.* 2016; **26:** 15-21.

Sakuta K, Saji N, Aoki J, Sakamoto Y, Shibazaki K, Iguchi Y, Kimura K. Decrease of Hyperintense Vessels on Fluid-Attenuated Inversion Recovery Predicts Good Outcome in t-PA Patients. *Cerebrovasc Dis.* 2016; **41:** 211-8.

Sakuta K, Iguchi Y, Sato T, Sakai K, Terasawa Y, Mitsumura H. Chronic kidney disease is independently associated with acute recurrent cerebral infarct in patients with atrial fibrillation. *J Clin Neurosci.* 2017 Jun; **40:** 97-101. Epub 2017 Mar 9.

Komatsu T, Mitsumura H, Yuki I, Iguchi Y. Primary Sjögren's syndrome presenting with multiple aneurysmal dilatation of cerebral arteries and causing repetitive intracranial hemorrhage. *J Neurol Sci.* 2016; **365:** 124-5.

Komatu T, Mitsumura H, Matsushima S, Iguchi Y. Migrating susceptibility vessel sign in posterior circulation stroke. *Am J Med.* 2016; **129:** e135-6.

Komatsu T, Terasawa Y, Arai A, Sakuta K, Mitsumura H, Iguchi Y. Transcranial color-coded sonography of vertebral artery for diagnosis of right-to-left shunts. *J Neurol Sci.* 2017; **376:** 97-101.

Sato T, Umehara T, Nakahara A, Oka H. Relative adrenal insufficiency in adult-onset cerebral X-linked adrenoleukodystrophy. *Neurology: Clinical Practice*. under publication. Epub 2016 Oct 2017.

Yamashita T, Miki A, Goto K, Araki S, Takizawa G, Ieki Y, Kiryu J, Tabuchi A, Iguchi Y, Kimura K, Yagita Y. Retinal ganglion cell atrophy in homonymous hemianopia due to acquired occipital lesions observed using Cirrus high-definition OCT. *Journal of Ophthalmology.* 2016; **9:** 1-9.

Saji N, Kimura K, Tateishi Y, Fujimoto S, Kaneko N, Urabe T, Tsujino A, Iguchi Y. Safety and efficacy of non-vitamin K oral anticoagulant treatment compared with warfarin in patients with non-valvular atrial fibrillation who develop acute ischemic stroke or transient ischemic attack: a multicenter prospective cohort study (daVinci study). *Journal of Thrombosis and Thrombolysis.* 2016; **42:** 453-62.

Yuan JH, Hashiguchi A, Yoshimura A, Yaguchi H, Tsuzaki K, Ikeda A, Wada-Isoe K, Ando M,

Nakamura T, Higuchi Y, Hiramatsu Y, Okamoto Y, Takashima H. Clinical diversity caused by novel IGHMBP2 variants. *J Hum Genet.* 2017 Jun; **62:** 599–604. Epub 2017 Mar 9.

Takagi S, Kono Y, Nagase M, Mochio S, Kato F. Facilitation of distinct inhibitory synaptic inputs by chemical anoxia in neurons in the oculomotor, facial and hypoglossal motor nuclei of the rat. *Exp Neurol.* 2017; **290:** 95–105.

Yamada S, Oikawa S, Komatsu T, Hrai T, Dohi K, Ogawa T. Early initiation of steroid pulse therapy for neuromyelitis optica in an emergency room setting. *AMS.* 2016; **3:** 171–3.

Department of Internal Medicine
Division of Nephrology and Hypertension

Takashi Yokoo, *Professor*
Keitaro Yokoyama, *Associate Professor*
Masato Ikeda, *Associate Professor*
Nobuo Tsuboi, *Assistant Professor*
Yukio Maruyama, *Assistant Professor*
Yoichi Miyazaki, *Professor*
Makoto Ogura, *Associate Professor*
Kazushige Hanaoka, *Assistant Professor*
Ichiro Ohkido, *Assistant Professor*

General Summary

Our department is one of the largest nephrology departments in Japan and includes all subspecialties of nephrology, i.e., from early chronic kidney disease (CKD) with proteinuria to dialysis and kidney transplantation. Therefore, our research groups are investigating diverse subjects and aim to eventually find new therapeutic strategies and mechanisms of disease progression, which may help decrease the number of patients with end-stage renal diseases.

Research Activities

Studies on IgA nephropathy (IgAN)
A multicenter, prospective cohort study (J-IGACS) is currently in progress. By using a national survey database (JRBR), we have conducted a study on regional variations of the clinical features at diagnostic renal biopsy in Japanese patients with IgA nephropathy.

Studies on low glomerular density (GD) in CKD
We have reported on glomerular density and volume in renal biopsies of children with proteinuria relative to preterm birth and gestational age (Koike K et al. Clin J Am Soc Nephrol. 2017). Collaborative research about the estimation of nephron numbers in Japanese is currently in progress.

Impact of hypertension, diabetes and aging on renal damage
Studies on renal alteration caused by hypertension, diabetes and aging using autopsy kidneys are currently in progress.

Studies on podocyte damage
Micro-array analysis of damaged podocytes in in vitro and in vivo revealed that ratios of increase in some 100 transcripts showed quite strong correlation between the two situations. Among these, Egr1 and Maff were included, transcription factors that antagonize Wt1 and Mafb, respectively. Indeed, level of podocin mRNA was dysregulated by a change in Egr1 or Maff expression. The result suggested that these two transcription factors can have an important role for the development of podocyte damage.

Studies of CKD-MBD
We previously reported that the DNA methylation patterns in CaSR and VDR genes were modified in the parathyroid glands (PTGs) of chronic kidney disease-mineral and bone disorder (CKD-MBD) (Uchiyama T et al. Hum Cell 2016). We then analyze the effect of histone modification in the PTGs of CKD-MBD. Furthermore, we are investigating how glial cells missing 2 (Gcm2) in PTGs, which is the essential transcription factor for parathyroid development in terrestrial vertebrates, affects PTGs function.
Magnesium (Mg) concentration is a proven predictor of mortality in hemodialysis patients. Judging from these facts, we showed that proton pump inhibitor use is associated with an increased risk of hypomagnesemia in hemodialysis patients by prospective cohort study (Nakashima A et al. PLOS ONE 2015).

Study of renal transplantation
We participated in Japan Academic Consortium of Kidney Transplantation (JACK) and published the following clinical and pathological analysis focused on; 1. HSPN(Kawabe M et al. CEN case Reports. 2016), 2. The prognostic value of Caveolin-1 in peritubular capillary(Nakada Y et al. Clinical Transplant. 2016) and 3. The significance of medullary ray injury(Niikura T et al. Transplant Proc. 2017).

Studies of peritoneal dialysis
We reported the change in clinical form of PD-associated peritonitis during 36 years and the difference of calcium and PTH levels PD and HD. We conduct clinical research of bicarbonate/lactate-buffered neutral PD solution, diabetic PD patients, and peritoneal membrane pathology.

Studies of anemia in CKD
We reported the association between higher serum ferritin and higher mortality among 191,902 HD patients using Japanese nationwide dialysis registry, We continue the clinical research among hepcidin, an important regulator of iron homeostasis, and clarified the clinical utility among non-dialyzed CKD patients.

Renal protective effects of T-type calcium channel blockade via blood brain barrier in chronic kidney disease model rats
We investigate whether there is different mechanism of renal protective effect via the agent's difference from capacity of penetrating the blood-brain barrier, using the new T-CCB agent, which can or cannot penetrate the blood-brain barrier.

Renal protective effects of azilsartan in adenine-induced renal failure model rats
Although daily urinary sodium excretion is decreased in non-medication group, azilsartan (Azi) suppressed the decreasing sodium excretion, urinary protein excretion and sympathetic nerve activity and upregulated renal ACE2 activity. We will investigate further molecular mechanism of renal protection.

Significance of serum uric acid level in patients receiving dialysis

In hemodialysis patients, lower levels of serum uric acid (SUA) were independently associated with all-cause and cardiovascular mortality among hemodialysis patients. However, in peritoneal dialysis patients, there are no relationship between SUA and mortality. Close monitoring of SUA is thought to be necessary for the management of hemodialysis patients.

Basic study for kidney regeneration

A novel system to regenerate the kidney by replacing nephron progenitor cells in an empty niche.

The kidneys develop through reciprocal and sequential interactions between the ureteric bud (UB) and surrounding cap mesenchyme (CM). The engraftment efficiency of cells transplanted to a nephrogenic niche has been very low, with the underlying cause considered to be the competition with the existing native host cells occupying the niche.

We demonstrated that the transplanted progenitor cells replaced the native progenitor cells in CM using a nephron progenitor eliminate system that used Cre-LoxP technology in combination with diphtheria toxin (DT)-mediated cell elimination.

Using the progenitor eliminate system, it was shown that competing native progenitor cells were completely replaced by transplant cells in CM. Furthermore, the replaced transplant cells displayed reciprocal interactions with the host UB and complete differentiation to nephrons.

Publications

Yamanaka S, Yokote S, Yamada A, Katsuoka Y, Izuhara L, Shimada Y, Omura N, Okano HJ, Ohki T, Yokoo T. Correction: Adipose Tissue-Derived Mesenchymal Stem Cells in Long-Term Dialysis Patients Display Downregulation of PCAF Expression and Poor Angiogenesis Activation. *PLoS One.* 2016; **11:** e0157282.

Nakada Y, Yamamoto I, Horita S, Kobayashi A, Mafune A, Katsumata H, Yamakawa T, Katsuma A, Kawabe M, Tanno Y, Ohkido I, Tsuboi N, Yamamoto H, Okumi M, Ishida H, Yokoo T, Tanabe K. The prognostic values of caveolin-1 immunoreactivity in peritubular capillaries in patients with kidney transplantation. *Clin Transplant.* 2016; **30:** 1417-24.

Morishita M, Matsuo N, Maruyama Y, Nakao M, Yamamoto I, Tanno Y, Ohkido I, Ikeda M, Yokoyama K, Yokoo T. The differences in acid-base status and the calcium parathyroid axis between peritoneal dialysis and hemodialysis. *Clin Nephrol.* 2016; **86:** 55-61.

Nakao M, Yamamoto I, Maruyama Y, Nakashima A, Matsuo N, Tanno Y, Ohkido I, Ikeda M, Yamamoto H, Yokoyama K, Yokoo T. 33 Years of Peritoneal Dialysis-Associated Peritonitis: A Single-Center Study in Japan. *Ther Apher Dial.* 2016; **20:** 60-5.

Uchiyama T, Tatsumi N, Kamejima S, Waku T (Doshisha Univ), Ohkido I, Yokoyama K, Yokoo T, Okabe M. Hypermethylation of the CaSR and VDR genes in the parathyroid glands in chronic kidney disease rats with high-phosphate diet. *Human Cell.* 2016; **29:** 155-61.

Kanzaki G, Nagasaka S[1], Higo S[1], Kajimoto Y[1], Kanemitsu T[1], Aoki M[1], Nagahama K[1], Natori Y (Iwate Medical Univ), Tsuboi N, Yokoo T, Shimizu A[1] ([1]Nippon Medical School). Impact of anti-glomerular basement membrane antibodies and glomerular neutrophil activation on glomerulonephritis in experimental myeloperoxidase-anti-neutrophil cytoplasmic antibody vasculitis. *Nephrol Dial Transplant.* 2016; **31:** 574-85.

Yokoyama K, Kurita N[1], Fukuma S (Kyoto Univ Hosp), Akizawa T (Showa Univ), Fukagawa M (Tokai Univ), Onishi Y (Institute for Health Outcomes and Process Evaluation Research), Kurokawa K (National Graduate Institute), Fukuhara S[1] ([1]Fukushima Medical Univ). Frequent monitoring of mineral metabolism in hemodialysis patients with secondary hyperparathyroidism: associations with achievement of treatment goals and with adjustments in therapy. *Nephrol Dial Transplant.* 2016; **32:** 534-41.

Tanaka M, Miyazaki Y, Koike K, Ueda H, Tsuboi N, Hirano K, Okonogi H, Ogura M, Kawamura T, Yokoo T. Clinicopathological char-

acteristics of patients with immunoglobulin A nephropathy showing acute exacerbations after favorable long-term clinical courses. *Clin Exp Nephrol.* 2016; **20:** 226-34.

Mafune A, Iwamoto T, Tsutsumi Y (Jichi Medical Univ), Nakashima A, Yamamoto I, Yokoyama K, Yokoo T, Urashima M. Associations among serum trimethylamine-N-oxide (TMAO) levels, kidney function and infarcted coronary artery number in patients undergoing cardiovascular surgery: a cross-sectional study. *Clin Exp Nephrol.* 2016; **20:** 761-9.

Okabayashi Y, Tsuboi N, Haruhara K, Kanzaki G, Koike K, Shimizu A, Miyazaki Y, Ohno I, Kawamura T, Ogura M, Yokoo T. Reduction of proteinuria by therapeutic intervention improves the renal outcome of elderly patients with IgA nephropathy. *Clin Exp Nephrol.* 2016; **20:** 910-7.

Katsuma A, Yamamoto I, Komatsuzaki Y, Niikura T, Kawabe M, Okabayashi Y, Yamakawa T, Katsumata H, Nakada Y, Kobayashi A, Tanno Y, Miki J, Yamada H, Ohkido I, Tsuboi N, Yamamoto H, Yokoo T. Subclinical antibody-mediated rejection due to anti-human-leukocyte-antigen-DR53 antibody accompanied by plasma cell-rich acute rejection in a patient with cadaveric kidney transplantation. *Nephrology (Carlton).* 2016; **Suppl 1:** 31-4.

Okabayashi Y, Yamamoto I, Komatsuzaki Y, Niikura T, Yamakawa T, Katsumata H, Kawabe M, Katsuma A, Nakada Y, Kobayashi A, Koike Y, Miki J, Yamada H, Tanno Y, Ohkido I, Tsuboi N, Ichida K, Yamamoto H, Yokoo T. Rare case of nephrocalcinosis in the distal tubules caused by hereditary renal hypouricaemia 3 months after kidney transplantation. *Nephrology (Carlton).* 2016; **Suppl 1:** 67-71.

Yamakawa T, Yamamoto I, Komatsuzaki Y, Niikura T, Okabayashi Y, Katsumata H, Kawabe M, Katsuma A, Mafune A, Nakada Y, Kobayashi A, Koike Y, Miki J, Yamada H, Tanno Y, Ohkido I, Tsuboi N, Yamamoto H, Yokoo T. Successful treatment of recurrent Henoch-Schönlein purpura nephritis in a renal allograft with tonsillectomy and steroid pulse therapy. *Nephrology (Carlton).* 2016; **Suppl 1:** 53-61.

Hirano K, Amano H, Kawamura T, Watanabe K, Koike K, Shimizu A, Endo S, Tsuboi N, Okonogi H, Miyazaki Y, Ikeda M, Hanaoka K, Ogura M, Komatsumoto S (Ashikaga Red Cross Hosp), Yokoo T. Tonsillectomy reduces recurrence of IgA nephropathy in mesangial hypercellularity type categorized by the Oxford classification. *Clin Exp Nephrol.* 2016; **20:** 425-32.

Kawabe M, Yamamoto I, Komatsuzaki Y, Yamakawa T, Katsumata H, Katsuma A, Mafune A, Nakada Y, Kobayashi A, Tanno Y, Ohkido I, Tsuboi N, Yokoyama K, Horita S[1], Okumi M[1], Ishida H[1], Yamamoto H, Yokoo T, Tanabe K[1] ([1]Tokyo Women's Medical Univ). Japan Academic Consortium of Kidney. Recurrence and graft loss after renal transplantation in adults with IgA vasculitis. *Clin Exp Nephrol*. Epub 2016 Sep 27. [Epub ahead of print]

Morisawa N, Koshima Y[1], Satoh JI[1] ([1]Saitama Red Cross Hosp), Maruyama Y, Kuriyama S, Yokoo T, Amemiya M. Usefulness of combination therapy with Daclatasvir plus Asunaprevir in chronic hepatitis C patients with chronic kidney disease. *Clin Exp Nephrol.* Epub 2016 Oct 22.

Ikeda M, Ueda Y, Maruyama Y, Yokoyama K, Yokoo T, Joki N (Toho Univ), Ando R (Musashino Red Cross Hosp), Shinoda T (Kawakita General Hosp), Inaguma D (Nagoya Daini Red Cross Hosp), Yamaka T (Kanagawa Institute of Technology), Komatsu Y (St. Luke's International Hosp), Koiwa F (Showa Univ), Sakaguchi T[1], Negi S[1], Shigematsu T[1] ([1]Wakayama Medical Univ). Possible prevention of uremic nausea by vitamin D receptor activators in non-dialysis patients with stage 5 chronic kidney disease. *Clin Exp Nephrol*. Epub 2016 Nov 14.

Nakao M, Yamamoto I, Maruyama Y, Morishita M, Nakashima A, Matsuo N, Tanno Y, Ohkido I, Ikeda M, Yamamoto H, Yokoyama K, Yokoo T. Risk factors for encapsulating peritoneal sclerosis: analysis of a 36-year experience in a University Hospital. *Nephrology (Carlton)*. Epub 2016 Aug 24.

Koike K, Ikezumi Y[1], Tsuboi N, Kanzaki G, Haruhara K, Okabayashi Y, Sasaki T, Ogura M, Saitoh A[1] ([1]Niigata Univ), Yokoo T. Glomerular Density and Volume in Renal Biopsy Specimens of Children with Proteinuria Relative to Preterm Birth and Gestational Age. *Clin J Am Soc Nephrol.* 2017; **12:** 585-90.

Haruhara K, Tsuboi N, Koike K, Kanzaki G, Okabayashi Y, Sasaki T, Fukui A, Miyazaki Y, Kawamura T, Ogura M, Yokoo T. Circadian blood pressure abnormalities in patients with primary nephrotic syndrome. *Clin Exp Hypertens.* 2017; **39:** 155-9.

Reviews and Books

Okabayashi Y, Tsuboi N, Sasaki T, Haruhara K, Kanzaki G, Koike K, Miyazaki Y, Kawamura T, Ogura M, Yokoo T. Glomerulopathy Associated With Moderate Obesity. *Kidney Int Rep.* 2016; **1:** 250-5.

Tsuboi N, Okabayashi Y, Shimizu A, Yokoo T. The renal pathology of obesity. *Kidney Int Rep.* 2017; **2:** 251-60.

Katsuma A, Yamakawa T, Nakada Y, Yamamoto I, Yokoo T. Histopathological findings in transplanted kidneys. *Renal Replacement Therapy.* 2017; **3:** 6. Epub 2017 Feb 16.

Komatsuzaki Y, Nakada Y, Yamamoto I, Kawabe M, Yamakawa T, Katsuma A, Mafune A, Kobayashi A, Koike Y, Yamada H, Miki J, Tanno Y, Ohkido I, Tsuboi N, Yokoyama K, Yamamoto H, Yokoo T. Successful Treatment of Plasma Cell-Rich Acute Rejection Using Pulse Steroid Therapy Alone: A Case Report. *Case Rep Transplant.* 2017; **2017:** 1347052.

Department of Internal Medicine
Division of Rheumatology

Daitaro Kurosaka, *Professor* Ken Yoshida, *Assistant Professor*

General Summary

An internist must aim to practice patient-oriented medicine that is well grounded in medical science. Therefore, our department encourages its staff members to do basic and clinical research. Major fields of research are clinical and experimental immunology.

Research Activities

We have performed clinical and experimental studies of rheumatic diseases.

1. Fasciitis in dermatomyositis

We have previously demonstrated that fasciitis is a common lesion of dermatomyositis detectable early after disease onset with *en bloc* biopsy and magnetic resonance imaging. Therefore, the detection of fasciitis plays an important role in the diagnosis of dermatomyositis, especially in its early stage. Power Doppler ultrasonography is useful for detecting inflammation and vascularity in rheumatic diseases. We showed that fasciitis is detected with power Doppler ultrasonography in patients with dermatomyositis and that angiogenesis is observed in fasciitis associated with dermatomyositis. This year, we have examined with immunohistochemical staining whether angiogenesis-related factors and inflammatory cytokines are expressed in the fascia.

2. Analysis of psychological tendency in patients with rheumatoid arthritis and a dissociation between disease activity and arthritic pain

Psychological factors are known to contribute to pain in motor disorders, in addition to localized inflammation. Therefore, through the use of a self-rating scale we evaluated depression and anxiety in patients with rheumatoid arthritis (RA). With a visual analogue scale as an indicator of pain and with the synovial blood flow signals as an indicator of synovitis, subjects were divided into 4 groups. We analyzed the associations between psychological tendency and arthritic pain in patients with RA. This year, we have examined the frequency and chacteristics of neuropathic-like pain in rheumatoid arthritis(RA) patients.

3. Citrullination of peptidylarginine deiminase in RA

Citrullination, catalysed by peptidylarginine deiminase (PAD), is a posttranslational modification of arginine to citrulline, which contributes to the pathogenesis of RA. We undertook a study to examine the presence and functions of citrullinated chemokines in RA. A newly developed enzyme-linked immunosorbent assay system showed that concentrations of citrullinated epithelial-derived neutrophil-activating peptide 78 (ENA-78)/chemokine (C-X-C motif) ligand 5 (CXCL5) were higher in synovial fluid from patients with RA than in synovial fluid from patients with other rheumatic diseases and correlated with the C-reactive protein level and the erythrocyte sedimentation rate. Although ENA-

78/CXCL5 is a neutrophil chemotactic factor, an *in-vitro* chemotaxis assay and *in-vivo* experiments showed that citrullinated ENA-78/CXCL5 has a monocyte-recruiting function and stimulates inflammation in an inflammatory arthritis model. Recently, autocitrullination of PAD has also been reported. In general, the enzyme activity of PAD is decreased after citrullination. However, the function of citrullinated PAD other than enzyme activity remains to be elucidated. This year, we investigated the functions of citrullinated PAD and noncitrullinated PAD about chemotaxis activity in vitro and arthritis-inducible activity in vivo.

4. *Bombina variegata* peptide 8/prokineticin 2 in RA

Prokineticin and its receptors are expressed in various tissues and are involved in diverse physiological functions, such as angiogenesis, neurogenesis, circadian rhythm, and the pain threshold. Of these functions, angiogenesis plays an important role in the pathogenesis of RA. We previously investigated prokineticin 2 expression in mice with collagen-induced arthritis, the animal model of RA, and reported that the expression of prokineticin 2 is significantly elevated in the joints of collagen-induced arthritis mice and correlates with the severity of arthritis. However, the mechanism of *Bombina variegata* peptide 8 regarding the onset of arthritis remained unknown. This year, we investigated the effect of an antagonist of prokineticin 2 on collagen-induced arthritis. Our data showed that administration of a prokineticin 2 antagonist suppressed the severity of arthritis. These results suggest that targeting prokineticin 2 provides a new therapeutic strategy for RA.

Publications

Yoshida K, Nishioka M, Matsushima S, Joh K, Oto Y, Yoshiga M et al. Power Doppler ultrasonography for detection of increased vascularity in the fascia: A potential early diagnostic tool in fasciitis of dermatomyositis. *Arthritis Rheumatol.* 2016; **68:** 2986-91.

Noda K, Yoshida K, Ukichi T, Furuya K, Hirai K, Kingetsu I, Kurosaka D. Myalgia in patients with dermatomyositis and polymyositis is attributable to fasciitis rather than myositis: a retrospective study of 32 patients who underwent histopathological examinations. *J Rheumatol.* 2017 Apr; **44:** 482-7. Epub 2017 Feb 1.

Ito H, Noda K, Yoshida K, Otani K, Yosiga M, Oto Y et al. Prokineticin 2 antagonist, PKRA7 suppresses arthritis in mice with collagen-induced arthritis. *BMC Musculoskelet Disord.* 2016; **8:** 387.

Chiba M, Yanaba K, Kohara A, Nakayama M, Nakagawa H, Fukuda T, Ishii N, Yoshida K. Septic arthritis caused by Mycobacterium marinum infection. *J Dermatol.* 2016 Nov; **14.** doi: 10.1111/1346-8138.13673. [Epub ahead of print]

Kohara A, Yanaba K, Muro Y, Ito H, Nakagawa H, Noda K, Kurosaka D. Anti-PM/Scl antibody-positive dermatomyositis in a Japanese patient: a case report and review of the literature. *Int J Rheum Dis.* 2017 Oct; **44:** 1179-80. Epub 2016 Nov 14.

Ito H, Noda K, Hirai K, Ukichi T, Furuya K, Kurosaka D. A case of systemic lupus erytematodes (SLE) following Human papillomavirus (HPV) vaccination. *Nihon Rinsho Meneki Gakkai Kaishi (Jpn J Clin Immunol)*. 2016; **39:** 145-9.

Department of Internal Medicine
Division of Cardiology

Michihiro Yoshimura, *Professor*
Teiichi Yamane, *Professor*
Shingo Seki, *Associate Professor*
Makoto Kawai, *Associate Professor*
Takayuki Ogawa, *Associate Professor*
Chikara Mori, *Assistant Professor*
Tomohisa Nagoshi, *Assistant Professor*
Ikuo Taniguchi, *Professor*
Kenichi Hongo, *Professor*
Takahiro Shibata, *Associate Professor*
Kimiaki Komukai, *Associate Professor*
Tetsuya Ishikawa, *Assistant Professor*
Kosuke Minai, *Assistant Professor*
Seiichiro Matsuo, *Assistant Professor*

General Summary

We have 6 research groups for covering the broad field of cardiology. In respective study groups, we have been studying the problems that face us in clinical practice. Our research is based on clinical studies that use the large database we have been developing. In specific, we recently used covariance structure analysis as a new solution for action assignments. Basic research is also performed to solve clinical questions.

Research Activities

Ischemic Heart Disease Research Group
We have converted patients' data, including risk factors and coronary lesion morphology, hemodynamic data, from cardiac catheterization examinations and treatments in patients with ischemic heart disease, into our large, precise database. Using this database, we have been performing a study comparing risk factors, clinical outcomes, and other data. We have recently reported the differences of risk factors between coronary organic stenosis and acute coronary syndrome by using covariance structure analysis. We have been using a similar method to study a contributing pattern of obesity to ischemic heart disease. In the analysis, we reported a possible risk of low-reactivity of natriuretic peptide. Furthermore, since fractional flow reserve is reportedly a good method for evaluating significant coronary stenosis, we have been collecting and analyzing information about the clinical data of fractional flow reserve (FFR).

Arrhythmia Research Group
In our arrhythmia team, we have been focusing on the curative treatment of atrial fibrillation. Our research activities include the comparison of efficiency and safety among different ablation methods (radiofrequency vs. cryoballoon ablation), factors associated with the occurrence of pulmonary vein stenosis following balloon ablation, and the association of ablation methods and asymptomatic cerebral ischemia.

Heart Failure Research Group
1. Study group on heart failure
Since last year, we have constructed and updated a database of approximately 3,000

patients who have been hospitalized for cardiac catheterization and treatment. Regarding this database, analysis is conducted using the Structural Equation Modeling (SEM) or Covariance Structure Analysis by adjusting the statistical analysis method, AMOS (Analysis of Moment Structures), which has been previously known but the use of which has been rarely reported in the cardiovascular field. In particular, we analyze the interrelationships of clinical factors that cannot be expressed by multivariate statistical analysis alone, focusing on statistical analysis research on clinical data including plasma BNP. Using a path diagram, it is easier to have a visual understanding of the relationships between each factor, and to perform multiple regression analysis and path analysis (repetition of multiple regression analysis) using factors and confirmatory factor analysis. Last year, with respect to the relationship between obesity and BNP, we reported the relationship between the change in BNP concentration before and after treatment along with the change in body weight. This spring, we published an article on the influence of remodeling changes in the left ventricular cavity on BNP concentration. By publishing articles on wide-ranging analysis results including detailed data analysis of the disease state of chronic heart failure along with the relationships between various valvular diseases and atrial fibrillation, we will continue to promote a wide range of clinical studies based on the experience gained from daily clinical practice. We are also continuing our efforts to clarify the mechanism of these findings via fundamental research.

Imaging Research Group

1. Study group on imaging

With the increasing number of cases involving transcatheter aortic valve replacement (TAVR) since last year, cardiac CT and echocardiograms are also vital as preoperative examinations for evaluating the aortic valve. From this valuable case information, we are seeking research agendas for clinical studies. Through other imaging modalities such as cardiac MRI and myocardial isotope tests, we are continuing to seek research agendas for clinical studies and conduct analyses on cardiomyopathy and arrhythmias.

Molecular Biology Research Group

Glucose becomes an important preferential substrate for cardiac metabolism and ATP generation during ischemia-reperfusion injury (IRI). Therefore, acceleration of glucose uptake and its metabolism is critical for myocardium to develop ischemic tolerance. Although insulin plays a pivotal role in this process, we have recently reported that insulin resistance increases during ischemic attack of acute coronary syndrome (ACS). The study also suggested that there are endogenous mechanisms of promoting glucose metabolism. One of the potential mechanisms is s sodium-glucose co-transporter 1 (SGLT1). A study of Langendorff murine heart perfusion demonstrated that the inhibition of SGLT1 during IRI reduces glucose uptake into the myocardium, leading to a decrease in the cardiac tissue ATP content. As a consequence, cardiac functional recovery after IRI was impaired by SGLT1-inhibition. The present findings provide the significant role of SGLT1 in optimizing cardiac energy metabolism during IRI.

Cardiac Physiology Research Group

We have demonstrated that thrombin, the final product of the coagulation cascade, in present in the heart. Coagulability is increased in patients with dilated cardiomyopathy (DCM). Using knock-in mice that have a cardiac troponin T deletion mutation that causes human DCM (ΔK210 knock-in mouse) (B6; 129-Tnnt2^{tm2Mmto}). We assessed the effects of a direct thrombin inhibitor, dabigatran, in ΔK210 knock-in mice. Dabigatran significantly improved fractional shortening in echocardiographic findings and survival outcomes. In conclusion, tissue thrombin is involved in the pathogenesis of DCM, and thrombin inhibition can be beneficial for the treatment of DCM.

To investigate this mechanism, we practiced microarray analysis, which has demonstrated that Casq1・Postn・Myh7 may be involved in the mechanism. We further practiced Western blot analysis, but there were no significant differences between hearts of DCM mice and Wild type mice in these three gene protein products. We next investigated the apoptosis by the TUNEL assay. The apoptotic index, the percentage of TUNEL-positive nuclei, was significantly increased in the DCM group in comparison to that observed in the Wild group. Treatment with dabigatran significantly reduced the apoptotic index. The apoptosis may be involved in the mechanism.

Publications

Mizuno Y*[1]*, Hokimoto S*[2]*, Harada E*[1]*, Kinoshita K*[3]*, Nakagawa K*[2]*, Yoshimura M, Ogawa H*[2]*, Yasue H*[1] *([1]Kumamoto Kinoh Hosp, [2]Kumamoto Univ, [3]Mukogawa Women's Univ). Variant aldehyde dehydrogenase 2 (ALDH2*2) is a risk factor for coronary spasm and ST-segment elevation myocardial infarction. *J Am Heart Assoc.* 2016; **5:** pii: e003247.

Kosuga T, Komukai K, Miyanaga S, Kubota T, Nakata K, Suzuki K, Yamada T, Yoshida J, Kimura H, Yoshimura M. Diabetes is a predictor of coronary artery stenosis in patients hospitalized with heart failure. *Heart Vessels.* 2016; **31:** 671-6.

Kinoshita K, Kawai M, Minai K, Ogawa K, Inoue Y, Yoshimura M. Potent influence of obesity on suppression of plasma B-type natriuretic peptide levels in patients with acute heart failure: An approach using covariance structure analysis. *Int J Cardiol.* 2016; **215:** 283-90.

Nishizaki Y*[1]*, Shimada K*[1]*, Tani S*[2]*, Ogawa T, Ando J*[3]*, Takahashi M*[3]*, Yamamoto M, Shinozaki T*[3]*, Miyazaki T*[1]*, Miyauchi K*[1]*, Nagao K*[2]*, Hirayama A*[2]*, Yoshimura M, Komuro I*[3]*, Nagai R*[5]*, Daida H*[1] *([1]Juntendo Univ, [2]Nihon Univ, [3]Univ Tokyo, [4]Tokyo Takanawa Hosp, [5]Jichi Med Univ). Association between the docosahexaenoic acid to arachidonic acid ratio and acute coronary syndrome: a multicenter observational study. *BMC Cardiovasc Disord.* 2016; **16:** 143.

Natsuaki M*[1]*, Morimoto T*[2]*, Yamamoto E*[3]*, Shiomi H*[3]*, Furukawa Y*[4]*, Abe M*[5]*, Nakao K*[6]*, Ishikawa T, Kawai K*[7]*, Yunoki K*[8]*, Shimizu S*[9]*, Akao M*[5]*, Miki S*[10]*, Yamamoto M*[11]*, Okada H*[12]*, Hoshino K*[13]*, Kadota K*[14]*, Morino Y*[15]*, Igarashi K*[16]*, Tanabe K*[17]*, Kozuma K*[18]*, Kimura T*[3] *([1]Saiseikai Fukuoka General Hosp, [2]Hyogo Coll Med, [3]Kyoto Univ, [4]Kobe City Med Center General Hosp, [5]National Hosp Organization Kyoto Med Center, [6]Saiseikai Kumamoto Hosp, [7]Chikamori Hosp, [8]Osaka City General Hosp, [9]Mashiko Hosp, [10]Mitsubishi Kyoto Hosp, [11]Kimitsu Chuo Hosp, [12]Seirei Hamamatsu General Hosp, [13]Nagai Hosp, [14]Kurashiki Central Hosp, [15]Iwate Med Univ Hosp, [16]Hokkaido Social Insurance Hosp, [17]Mitsui Memorial Hosp, [18]Teikyo Univ Hosp). One-year outcome of a prospective trial stopping dual antiplatelet therapy at 3 months after everolimus-eluting cobalt-chromium stent implantation: ShortT and OPtimal duration of Dual AntiPlatelet Therapy after everolimus-eluting cobalt-chromium stent (STOPDAPT) trial. *Cardiovasc Interv Ther.* 2016; **31:** 196-209.

Tokuda M, Yamane T, Sadaoka S, Tokutake K, Yokoyama K, Hioki M, Narui R, Tanigawa S, Inada K, Matsuo S, Yoshimura M. Percutaneous retrieval of a radiolucent anchoring sleeve embolized in pulmonary artery during pacemaker implantation. *Heart Vessels.* 2016; **31:** 1402-4.

Kimura H, Ishikawa T, Nakata K, Kubota T, Komukai K, Yoshimura M. A case of recoiling of everoimus-eluting stent at the ostium of the right coronary artery by out-stent plaque progression. *Jikeikai Med J.* 2016; **63:** 71-6.

Tokutake K, Tokuda M, Ogawa T, Matsuo S, Yoshimura M, Yamane T. Pulmonary vein stenosis after second-generation cryoballoon ablation for atrial fibrillation. *HeartRhythm Case Rep.* 2016;

3: 36–9.

Tokuda M, Matsuo S, Isogai R, Uno G, Tokutake K, Yokoyama K, Kato M, Narui R, Tanigawa S, Yamashita S, Inada K, Yoshimura M, Yamane T. Adenosine testing during cryoballoon ablation and radiofrequency ablation of atrial fibrillation: A propensity score-matched analysis. *Heart Rhythm.* 2016; **13:** 2128–34.

Watanabe M[1], Saito Y[1], Aonuma K[2], Hirayama A[3], Tamaki N[4], Tsutsui H[4], Murohara T[5], Ogawa H[6], Akasaka T[7], Yoshimura M, Sato A[2], Takayama T[3], Sakakibara M[4], Suzuki S[5], Ishigami K[8], Onoue K[1]; CINC-J study investigators ([1]Nara Medical Univ, [2]Tsukuba Univ, [3]Nihon Univ, [4]Hokkaido Univ, [5]Nagoya Univ, [6]Kumamoto Univ, [7]Wakayama Med Univ, [8]Saiseikai-Suita Hosp). Prediction of contrast-induced nephropathy by the serum creatinine level on the day following cardiac catheterization. *J Cardiol.* 2016; **68:** 412–8.

Seki S, Oki Y, Tsunoda S, Takemoto T, Koyama T, Yoshimura M. Impact of alcohol intake on the relationships of uric acid with blood pressure and cardiac hypertrophy in essential hypertension. *J Cardiol.* 2016; **68:** 447–54.

Hioki M, Matsuo S, Tokutake K, Yokoyama K, Narui R, Ito K, Tanigawa S, Tokuda M, Yamashita S, Anan I, Inada K, Sakuma T, Sugimoto K, Yoshimura M, Yamane T. Filling defects of the left atrial appendage on multidetector computed tomography: their disappearance following catheter ablation of atrial fibrillation and the detection of LAA thrombi by MDCT. *Heart Vessels.* 2016; **31:** 2014–24.

Takahashi M[1], Ando J[1], Shimada K[1], Nishizaki Y[2], Tani S[3], Ogawa T, Yamamoto M[4], Nagao K[3], Hirayama A[3], Yoshimura M, Daida H[2], Nagai R[5], Komuro I[1] ([1]Univ Tokyo, [2]Juntendo Univ, [3]Nihon Univ Hosp, [4]Sempo Takanawa Hosp, [5]Jichi Med Univ). The ratio of serum n-3 to n-6 polyunsaturated fatty acids is associated with diabetes mellitus in patients with prior myocardial infarction: a multicenter cross-sectional study. *BMC Cardiovasc Disord.* 2017; **17:** 41.

Mizuno Y[1], Hokimoto S[1], Harada E[1], Kinoshita K[2], Yoshimura M, Yasue H[1] ([1]Kumamoto Univ, [2]Mukogawa Women's Univ). Variant aldehyde dehydrogenase 2 (ALDH*2) in east asians interactively exacerbates tobacco smoking risk for coronary spasm — Possible role of reactive aldehydes. *Circ J.* 2017; **81:** 96–102.

Sato A[1], Aonuma K[1], Watanabe M[2], Hirayama A[3], Tamaki N[4], Tsutsui H[4], Murohara T[5], Ogawa H[6], Akasaka T[7], Yoshimura M, Takayama T[3], Sakakibara M[4], Suzuki S[5], Ishigami K[8], Onoue K[2], Saito Y[2] ([1]Univ Tsukuba, [2]Nara Med Univ, [3]Nihon Univ, [4]Hokkaido Univ, [5]Nagoya Univ, [6]Kumamoto Univ, [7]Wakayama Med Univ, [8]Saiseikai-Suita Hosp); CINC-J study investigators. Association of contrast-induced nephropathy with risk of adverse clinical outcomes in patients with cardiac catheterization: From the CINC-J study. *Int J Cardiol.* 2017; **227:** 424–9.

Miyamoto T[1], Ishikawa T, Nakano Y[1], Mutoh M ([1]Saitama Cardiovascular Respiratory Center). Very long-term clinical and angiographic outcomes after sirolimus- and paclitaxel-eluting stent placement for ST-elevation myocardial infarction: a propensity score-matched comparison. *Cardiovasc Interv Ther.* 2017; **32:** 24–35.

Ito K, Hongo K, Date T, Ikegami M, Hano H, Owada M, Morimoto S, Kashiwagi Y, Katoh D, Yoshino T, Yoshii A, Kimura H, Nagoshi T, Kajimura I, Kusakari Y, Akaike T, Minamisawa S, Ogawa K, Minai K, Ogawa T, Kawai M, Yajima J, Matsuo S, Yamane T, Taniguchi I, Morimoto S, Yoshimura M. Tissue thrombin is associated with the pathogenesis of dilated cardiomyopathy. *Int J Cardiol.* 2017; **228:** 821–7.

Ogawa K, Minai K, Kawai M, Tanaka T, Nagoshi T, Ogawa T, Yoshimura M. Parallel comparison of risk factors between progression of organic stenosis in the coronary arteries and onset of acute coronary syndrome by covariance structure analysis. *PLoS One.* 2017; **12:** e0173898.

Department of Internal Medicine
Division of Diabetes, Metabolism and Endocrinology

Kazunori Utsunomiya, *Professor*
Kuninobu Yokota, *Professor*
Masami Nemoto, *Associate Professor*
Rimei Nishimura, *Associate Professor*
Kousai Sano, *Assistant Professor*
Masaya Sakamoto, *Assistant Professor*
Sho Ishizawa, *Assistant Professor*
Katsunori Tojo, *Professor*
Yutaka Mori, *Professor*
Tamotsu Yokota, *Associate Professor*
Shuichi Kato, *Assistant Professor*
Kei Fujimoto, *Assistant Professor*
Daiji Kawanami, *Assistant Professor*

General Summary

We see over 15,000 patients a month in our department, and the patients are increasing every years. Mainly we see diabetic patients (including 10% of Type1 diabetes), but our patients have multiple of disease like thyroid grand, pituitary gland and gonad.
Physicians should practice not only contribute to make advances of diabetes and endocrinology, but provide best healthcare to our patients which consists of research evidence, clinical expertise, and patients' preferences. To accomplish this goal, we encourage the members of our staff to do quality basic and clinical research. And we accept many students japan and abroad far more than our college. Futhermore, we educate foundation house officers and specialist trainees about positive conference presentation.

Research Activities

Diabetes complications
1. Molecular mechanisms governing intracellular signal transduction focusing on cell types relevant to diabetic complications
2. Roles of small GTP‐binding protein Rho and Rho‐kinase in renal, retinal, neuronal, and endothelial biology
3. Isoform‐specific roles of Rho‐kinase in pathogenesis of diabetes. Approaches to this study range from in vitro to in vivo using gene‐targeting approaches in mice

Epidemiology
1. Clinical trials of the treatment with diabetic patients using continuous glucose monitoring (CGM) and Flash Glucose Monitoring FGM)
2. A nationwide epidemiologic study of mortality in approximately 3,500 patients with type 1 diabetes was started in 1986 and has continued to provide much information about the prognosis of Japanese children with type 1 diabetes
3. A population‐based study of childhood obesity and insulin resistance as well as diabetes in elderly and genetic factors has also continued in Niigata Prefecture
4. Epidemiological study using data consisted of more than 6,000 individuals with diabetes from 4 Jikei University hospitals

Molecular biology for pancreatic islets
Basic research
Insulin insufficiency and insulin resistance are main cause of diabetes but yet to be elucidated. In addition to this, dysregulated glucagon secretion and function are considered as important as insulin, namely "bi-hormonal disorder". Although glucagon secretion is suppressed by insulin, dysregulated glucagon secretion is seen in diabetic patient.
Recently, insulin resistance in pancreatic alpha cell was proposed which results in dysregulated glucagon secretion leading to diabetes.
Serendipically, we found that serine/threonine kinase protein kinase c (PKC) delta is involved in alpha cells glucagon secretion. Thus, the aim of this study is to elucidate the mechanism of PKCdelta-dependent glucagon secretion in insulin resistant alpha cells. Ongoing projects are as following.
1. Insulin resistance for glucagon secretion is studied in glucagon-secreting alpha TC1 cell line
2. Involvement of PKCdelta is studied by chemically and genetically inhibition PKCdelta in alpha TC1 cell line
3. To elucidate the molecular mechanism of PKCdelta in vivo, we are establishing alpha cell specific PKCdelta knockout mice (αPKCδKO)
4. Establishment (confirmation) of alpha cell insulin resistance in vivo
5. Physiological and histological characterization of αPKCδKO in wild type and diabetic mice

Endocrinology
Basic research
1. The role of 12-lipoxygenase on diabetic cardiomyopathy
2. The role of BRS (baroreflex sensibility) on diabetic macroangiopathy especially effects of glycemic variability and blood pressure variability
3. Effect of SGLT-2 inhibitor in diabetic model rats
4. Effect of aldosterone in macula lutea degeneration

Clinical research
1. Effect of SGLT-2 inhibitor in diabetic patients
2. The role of BRS (baroreflex sensibility) on diabetic patients
3. The durability of basal insulin affects day-to-day glycemic variability assessed by continuous glucose monitoring in type 2 diabetes patients
4. Investitation of HbA1c variability in type 2 diabetic patients. (JDDM)
5. Achievement of HbA1c and Blood pressure and LDL-C goal of type diabetic patients (JDDM)

Publications

Tajima N, Nishimura R, Izumi K, Hayashino Y, Origasa H, Noda M, Ueki K. A large-scale, observational study to investigate the current status of diabetes complications and their prevention in Japan: research outline and baseline data for type 2 diabetes — JDCP study 1. *Diabetol Int.* 2015; **6:** 243-51.
Sasaki T, Seino Y, Fukatsu A, Ubukata M,

Sakai S, Samukawa Y. Pharmacokinetics, Pharmacodynamics, and Safety of Luseogliflozin in Japanese Patients with Type 2 Diabetes Mellitus: A Randomized, Single-blind, Placebo-controlled Trial. *Adv Ther.* 2015; **32:** 319-40.

Sasaki T, Seino Y, Fukatsu A, Ubukata M, Sakai S, Samukawa Y. Absence of Drug-Drug Interactions Between Luseogliflozin, a Sodium-Glucose Co-transporter-2 Inhibitor, and Various Oral Antidiabetic Drugs in Healthy Japanese Males. *Adv Ther.* 2015; **32:** 404-17.

Katoh S, Markku Peltonen, Zeniya M, Kaji M, Sakamoto Y, Utsunomiya K, Tuomilehto J. Analysis of the Japanese Diabetes Risk Score and fatty liver markers for incident diabetes in a Japanese cohort. *Prim Care Diabetes.* 2016; **10:** 19-26.

Nishimura R, Izumi K, Hayashino Y, Origasa H, Noda M, Ueki K, Tajima N. A large-scale observational study to investigate the current status of diabetes complications and their prevention in Japan: research outline and baseline data for type 1 diabetes JDCP study 2. *Diabetol Int.* 2016; **7:** 4-11.

Nishimura R, Omiya H, Sugio K, Ubukata M, Sakai S, Samukawa Y. Sodium-glucose cotransporter 2 inhibitor luseogliflozin improves glycaemic control, assessed by continuous glucose monitoring, even on a low-carbohydrate diet. *Diabetes Obes Metab.* 2016; **18:** 702-6.

Kano M, Takekoshi S, Osamura R, Tojo K, Utsunomiya K, Yamada S. Characterization of possible tumor stem cells from human pituitary adenomas. *Jikeikai Med J.* 2016; **63:** 15-25.

Tsukamoto M, Niimi N, Sango K, Takaku S, Kanazawa Y, Utsunomiya K. Neurotropic and neuroprotective properties of exendin-4 in adult rat dorsal root ganglions: involvement if insulin and RhoA. *Histochem Cell Biol.* 2015; **144:** 249-59.

Onda Y, Nishimura R, Morimoto A, Sano H, Utsunomiya K, Tajima N. For The Diabetes Epidemiology Research International (DERI) Mortality Study Group. Causes of death in patients with childhood-onset type 1 diabetes receiving dialysis in Japan: Diabetes Epidemiology Research International (DERI) Mortality Study. *Diabetes and its complications.* 2015; **29:** 903-7.

Onda Y, Nishimura R, Morimoto A, Sano H, Utsunomiya K, Tajima N. For The Diabetes Epidemiology Research International (DERI) Mortality Study Group. Age at Transition from Pediatric to Adult Care Has No Relationship with Mortality for Childhood-Onset Type 1 Diabetes in Japan: Diabetes Epidemiology Research International (DERI) Mortality Study. *PLosOne.* 2016; **11:** e0150720.

Iuchi H, Sakamoto M, Suzuki H, Kayama Y, Ohashi K, Hayashi T, Ishizawa S, Yokota T, Tojo K, Yoshimura M, Utsunomiya K. Effect of One-Week Salt Restriction on Blood Pressure Variability in Hypertensive Patients with Type 2 Diabetes. *PLoSOne.* 2016; **11:** e0144921.

Ohashi K, Hayashi T, Sakamoto M, Iuchi H, Suzuki H, Ebisawa T, Tojo K, Sasano H, Utsunomiya K. Aldosterone-producing adrenocortical carcinoma with prominent hepatic metastasis diagnosed by liver biopsy: a case report. *BMC Endocr Disord.* 2016; **16:** 3.

Mitsuishi S, Nishimura R, Ando K, Tsujino D, Utsunomiya K. Can Fasting Glucose Levels or Post-Breakfast Glucose Fluctuations Predict the Occurrence of Nocturnal Asymptomatic Hypoglycemia in Type 1 Diabetic Patients Receiving Basal-Bolus Insulin Therapy with Long-Acting Insulin? *PLoSOne.* 2015; **10:** e0144041.

Takeda Y, Kawanami D, Utsunomiya K. Detection of hemoglobin variant HbS on the basis of discrepant HbA1c values in different measurement methods. *Diabetol Int.* 2016; **7:** 199-203.

Ochiai H, Shirasawa T, Nanri H, Nishimura R, Matoba M, Hoshino H, Kokaze A. Eating quickly is associated with waist-to-height ratio among Japanese adolescents: a cross-sectional survey. *Arch Public Health.* 2016; **74:** 18.

Samukawa Y, Omiya H, Watase H, Nozaki K, Sakai S, Nishimura R. Substantial Effects of Luseogliflozin Revealed by Analyzing Responses to Postprandial Hyperglycemia: Post Hoc Subanalyses of a Randomized Controlled Study. *Adv Ther.* 2016; **33:** 1215-30.

Sone H, Nakagami T, Nishimura R, Tajima N; MEGA Study Group. Comparison of lipid parameters to predict cardiovascular events in Japanese mild-to-moderate hypercholesterolemic patients with and without type 2 diabetes: Subanalysis of the MEGA Study. *Diabetes Res Clin Pract.* 2016; **113:** 14-22.

Shirasawa T, Ochiai H, Nanri H, Nishimura R, Ohtsu T, Hoshino H, Tajima N, Kokaze A. The relationship between distorted body image and lifestyle among Japanese adolescents: a population-based study. *Arch Public Health.* 2015; **73:** 32.

Haneda M, Seino Y, Inagaki N, Kaku K, Sasaki T, Fukatsu A, Kakiuchi H, Sato Y, Sakai S, Samukawa Y. Influence of Renal Function on the 52-Week Efficacy and Safety of the Sodium Glucose Cotransporter 2 Inhibitor Luseogliflozin in Japanese Patients with Type 2 Diabetes Mellitus. *Clin Ther.* 2016; **38:** 66-88.

Seino Y, Inagaki N, Haneda M, Kaku K, Sasaki T, Fukatsu A, Ubukata M, Sakai S, Samukawa Y. Efficacy and safety of luseogliflozin added to various oral antidiabetic drugs in Japanese patients with type 2 diabetes mellitus. *J Diabetes Investig.* 2015; **6:** 443-53.

Department of Internal Medicine
Division of Clinical Oncology/Hematology

Keisuke Aiba, *Professor*
Takaki Shimada, *Associate Professor*
Hidekazu Masuoka, *Assistant Professor*
Shingo Yano, *Assistant Professor*
Yoji Ogasawara, *Assistant Professor*
Takeshi Saito, *Assistant Professor*
Noriko Usui, *Apointed Profesor*
Nobuaki Dobashi, *Associate Professor*
Kaichi Nishiwaki, *Assistant Professor*
Yuhichi Yahagi, *Assistant Professor*
Katsuki Sugiyama, *Assistant Professor*
Yuko Shiota, *Assistant Professor*

General Summary

The immediate goals of our clinical and basic research are to investigate basic and clinical aspects of malignant diseases and to try to improve outcomes for patients with solid tumors and hematological malignancies, leading to the ultimate goals of improving the natural history of malignant diseases. We have also been performing several clinical trials and basic research studies successfully throughout 2016.

Research Activities

Leukemias
Many patients with previously untreated hematological disorders have been referred to our department. The disorders in 2016 included acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL), 24 cases and chronic myeloid leukemia (CML), 4 cases. We have performed clinical trials as a member of the Japan Adult Leukemia Study Group (JALSG), which is a distinguished leukemia research group established more than 20 years ago in Japan for clinical research and treatment of AML, ALL, and CML.

Lymphomas
In 2016 we registered 67 patients with newly diagnosed non-Hodgkin's lymphoma. We have performed clinical trials as a member of the Lymphoma Study Group of the Japan Clinical Oncology Group (JCOG). The study JCOG0601 (newly diagnosed low risk advanced diffuse large B cell lymphoma: phase II/III) was pivotal protocol studies beginning in 2007.

Myeloma
We registered 9 patients with newly diagnosed multiple myeloma in 2016. A novel agent, the proteasome inhibitor bortezomib, became available in 2007, and we have used it with or without dexamethasone to treat patients who had refractory myeloma.
In-house protocols are also under investigation. A phase II study of CVD regimen (cyclo phosphamide+bortezomib+dexamethasone) for patients with newly diagnosed multiple myeloma.

Hematopoietic stem cell transplantation

To investigate and establish safer and more effective hematopoietic stem cell transplantation (HSCT), we have performed serial clinical studies examining umbilical cord blood transplantation, reduced-intensity stem cell transplantation from haploidentical donor, and investigation of mechanisms of graft-versus-host disease in HSCT.

Solid tumors

Many patients with solid cancers have been referred to our department from related divisions or departments from both inside and outside our hospital. Several of our studies seeking improved therapeutic outcomes are in progress throughout our university hospital with related divisions or departments. Since late 2008 we have been investigating a combined-modality therapy of radiation and chemotherapy with docetaxel, cisplatin, and 24 hours' continuous infusion of 5-FU (DCF regimen) for patients with locally advanced esophageal cancer. The study has been completed and the results have published recently. Successively an improved protocol was launched 3 years ago and now has been investigating. We performed a novel drug-development study with an orally decaying formulation of S-1 co-operating with a colleague department had completed in patients with advanced gastric cancer and the new formulation of S-1 became now available in daily practice. Our first-line chemotherapies for patients with advanced colorectal cancer are folinic acid, fluorouracil, and oxaliplatin (FOLFOX) and folinic acid, 5-FU, and irinotecan (FOLFIRI). Since antibodies against vascular endothelial growth factor (VEGF) and against epidermal growth factor receptor (EGFR) became available in 2007 and 2008, respectively, combination therapies of these antibodies and FOLFOX or FOLFILI have also been performed. Since oral drugs are more convenient and safer, 5-FU is replaced by S-1 or capecitabine in such i.v. combination chemotherapy as FOLFOX or FOLFILI, leading to develop improved regimens of SOX, XELOX, IRIS and XELIRI. Salvage therapies using regorafenib or TAS-102 became standard care for resistant and refractory advanced colorectal cancer.

Basic research

One of our important activities is translational research on solid cancers and hematological malignancies. Since clinical requirement is urgent, persistent research is warranted. Cancer fatigue is now an emerging issue for patients with advanced malignant disease. We have been evaluating the correlation between cancer fatigue and human herpesvirus 6 (HHV-6) reactivation using patient's salivary juice and blood samples, collaborating with the department of Virology. The preliminary result was reported at the annual meeting of the Multinational Association of Supportiv Care in Cancer (MASCC) in Miami, Florida (USA).

Life- threatening disease, such a study seems to be highly of great consequence. Supportive care in cancer is also very important for patients with malignant disease. We have been working on such a field for years. Measuring L-FABP level in patient's urine can predict renal damage caused by cisplatin. Therefore, we have been trying to see if L-FABP is worth enough to measure for early detection of renal damage in patients undergoing cisplatin combination chemotherapy such as DCF and GDP. The studies are

vigorously in progress.

Publications

Aoki J, Seo S, Kanamori H, Tanaka M, Fukuda T, Onizuka M, Kobayashi N, Kondo T, Sawa M, Uchida N, Iwato K, Icihnohe T, Atsuta Y, Yano S, Takami A. Impact of low-dose TBI on outcomes of reduced intensity conditioning allogeneic hematopoietic stem cell transplantation for AML. *Bone Marrow Transplant.* 2016; **51:** 604-6.

Aoki J, Kanamori H, Tanaka M, Yamasaki S, Fukuda T, Ogawa H, Iwato K, Ohashi K, Okumura H, Onizuka M, Maesako Y, Teshima T, Kobayashi N, Morishima Y, Hirokawa M, Atsuta Y, Yano S, Takami A. Impact of age on outcomes of allogeneic hematopoietic stem cell transplantation with reduced intensity conditioning in elderly patients with acute myeloid leukemia. *Am J Hematol.* 2016; **91:** 302-7.

Kurosawa S, Yamaguchi H, Yamaguchi T, Fukunaga K, Yui S, Wakita S, Kanamori H, Usuki K, Uoshima N, Yanada M, Shono K, Ueki T, Mizuno I, Yano S, Takeuchi J, Kanda J, Okamura H, Inamoto Y, Inokuchi K, Fukuda T. Decision Analysis of Postremission Therapy in Cytogenetically Intermediate-Risk Acute Myeloid Leukemia: The Impact of FLT3 Internal Tandem Duplication, Nucleophosmin, and CCAAT/Enhancer Binding Protein Alpha. *Biol Blood Marrow Transplant.* 2016; **22:** 1125-32.

Yanada M, Yano S, Kanamori H, Gotoh M, Emi N, Watakabe K, Kurokawa M, Nishikawa A, Mori T, Tomita N, Murata M, Hashimoto H, Henzan H, Kanda Y, Sawa M, Kohno A, Atsuta Y, Ichinohe T, Takami A. Autologous hematopoietic cell transplantation for acute promyelocytic leukemia in second complete remission: outcomes before and after the introduction of arsenic trioxide. *Leuk Lymphoma.* 2017; **58:** 1061-7.

Suzuki K, Yano S, Nishiwaki K, Sano K, Shimada T, Yahagi Y, Ogasawara Y, Sugiyama K, Takahara S, Saito T, Kasama K, Minami J, Yokoyama H, Kamiyama Y, Katsube A, Masuoka H, Katori M, Machishima T, Ouchi A, Dobashi N, Kaito K, Usui N, Aiba K. Clinical significance of granule-containing myeloma cells in patients with newly diagnosed multiple myeloma. *Cancer Med.* 2016; **5:** 3051-8.

Hagihara M, Iriyama N, Yoshida C, Wakita H, Chiba S, Okamoto S, Kawakami K, Takezako N, Kumagai T, Inokuchi K, Ohyashiki K, Taguchi J, Yano S, Igarashi T, Kouzai Y, Morita S, Sakamoto J, Sakamaki H. Association of pleural effusion with an early molecular response in patients with newly diagnosed chronic-phase chronic myeloid leukemia receiving dasatinib: Results of a D-First study. *Oncol Rep.* 2016; **36:** 2976-82.

Takagi S, Masuoka K, Uchida N, Kurokawa M, Nakamae H, Imada K, Iwato K, Ichinohe T, Atsuta Y, Takami A, Yano S. Allogeneic Hematopoietic Cell Transplantation for Leukemic Transformation Preceded by Philadelphia Chromosome-Negative Myeloproliferative Neoplasms: A Nationwide Survey by the Adult Acute Myeloid Leukemia Working Group of the Japan Society for Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant.* 2016; **22:** 2208-13.

Yamasaki S, Hirakawa A, Aoki J, Uchida N, Fukuda T, Ogawa H, Ohashi K, Kondo T, Eto T, Kanamori H, Okumura H, Iwato K, Ichinohe T, Kanda J, Onizuka M, Kuwatsuka Y, Yanada M, Atsuta Y, Takami A, Yano S. Role of reduced-intensity conditioning allogeneic hematopoietic cell transplantation in older patients with de novo acute myeloid leukemia. *Ann Hematol.* 2017; **96:** 289-97.

Konuma T, Kondo T, Yamashita T, Uchida N, Fukuda T, Ozawa Y, Ohashi K, Ogawa H, Kato C, Takahashi S, Kanamori H, Eto T, Nakaseko C, Kohno A, Ichinohe T, Atsuta Y, Takami A, Yano S; Adult Acute Myeloid Leukemia Working Group of the Japan Society for Hematopoietic Cell Transplantation (JSHCT). Outcome of allogeneic hematopoietic stem cell transplantation in adult patients with acute myeloid leukemia harboring trisomy 8. *Ann Hematol.* 2017; **96:** 469-78.

Yanada M, Kurosawa S, Kobayashi T, Ozawa Y, Kanamori H, Kobayashi N, Sawa M, Nakamae H, Uchida N, Hashimoto H, Fukuda T, Hirokawa M, Atsuta Y, Yano S. Reduced-intensity conditioning allogeneic hematopoietic cell transplantation for younger patients with acute myeloid leukemia: a registry-based study. *Bone Marrow Transplant.* 2017 Jun; **52:** 818-24. Epub 2017 Jan 23.

Mashima K, Yano S, Yokoyama H, Saito T, Machishima T, Shimada T, Yahagi Y, Takahara S, Sugiyama K, Ogasawara Y, Minami J, Kamiyama Y, Katsube A, Suzuki K, Ohshima S, Yamada H, Usui N, Aiba K. Epstein-Barr Virus-associated Lymphoproliferative Disorder with Encephalitis Following Anti-thymocyte Globulin for Aplastic Anemia Resolved with Rituximab Therapy: A Case Report and Literature Review. *Intern Med.* 2017; **56:** 701-6.

Hayashi K, Nagasaki E, Kan S, Ito M, Kamata Y, Homma S, Aiba K. Gemcitabine enhances rituximab-mediated complement-dependent cytotoxicity to B cell lymphoma by CD20 upregulation. *Cancer Sci.* 2016; **107:** 682-9.

Shirai Y, Shiba H, Iwase R, Haruki K, Fujiwara Y, Furukawa K, Uwagawa T, Ohashi T, Yanaga K. Dual inhibition of nuclear factor kappa-B and Mdm2 enhance the antitumor effect of radiation therapy for pancreatic cancer. *Cancer Lett.* 2016;

370: 177–84.
Yang S, He P, Wang J, Schetter A, Tang W, Funamizu N, Yanaga K, Uwagawa T, Satoskar AR, Gaedcke J, Bernhardt M, Ghadimi BM, Gaida MM, Bergmann F, Werner J, Ried T, Hanna N, Alexander HR, Hussain SP. A Novel MIF Signaling Pathway Drives the Malignant Character of Pancreatic Cancer by Targeting NR3C2. *Cancer Res.* 2016; **76:** 3838–50.
Horiuchi T, Uwagawa T, Shirai Y, Saito N, Iwase R, Haruki K, Shiba H, Ohashi T, Yanaga K. New treatment strategy with nuclear factor-κB inhibitor for pancreatic cancer. *J Surg Res.* 2016; **206:** 1–8.
Fukushima R, Kobayashi Y, Fukuhara S, Miyamoto K, Munakata W, Maruyama D, Kim SW, Watanabe T, Taniguchi H, Maeshima A, Tobinai K. A retrospective analysis of combination chemotherapy consisting cyclophosphamide, vincristine, prednisolone and procarbazine (C-MOPP) for pretreated aggressive non-Hodgkin lymphoma. *J Chemother.* 2016; **28:** 116–22.
Ishii H, Konuma T, Kato S, Oiwa-Monna M, Tojo A, Takahashi S. Impact of hematogones on the long-term outcomes of single-unit cord blood transplantation for adult patients. *Leuk Lymphoma.* 2017; **58:** 118–126.
Miyasaka N, Miura O, Kawaguchi T, Arima N, Morishita E, Usuki K, Morita Y, Nishiwaki K, Ninomiya H, Gotoh A, Imashuku S, Urabe A, Shichishima T, Nishimura J, Kanakura Y. Pregnancy outcomes of patients with paroxysmal nocturnal hemoglobinuria treated with eculizumab: a Japanese experience and updated review. *Int J Hematol.* 2016; **103:** 703–12.
Kawamura K, Ikeda T, Hagiwara S, Mori T, Shinagawa A, Nishiwaki K, Ohashi K, Kubonishi S, Fukuda T, Ito T, Tomita N, Ichinohe T, Kato K, Morishima Y, Atsuta Y, Sunami K, Kanda Y. Tandem autologous versus autologous/allogeneic transplantation for multiple myeloma: propensity score analysis. *Leuk Lymphoma.* 2016; **57:** 2077–83.
Ninomiya H, Obara N, Chiba S, Usuki K, Nishiwaki K, Matsumura I, Shichishima T, Okamoto S, Nishimura JI, Ohyashiki K, Nakao S, Ando K, Kanda Y, Kawaguchi T, Nakakuma H, Harada D, Akiyama H, Kinoshita T, Ozawa K, Omine M, Kanakura Y. Interim analysis of post-marketing surveillance of eculizumab for paroxysmal nocturnal hemoglobinuria in Japan. *Int J Hematol.* 2016; **104:** 548–58.

Department of Internal Medicine
Division of Respiratory Diseases

Kazuyoshi Kuwano, *Professor*
Katsutoshi Nakayama, *Associate Professor*
Masamichi Takagi, *Assistant Professor*
Takanori Numata, *Assistant Professor*
Akira Kojima, *Professor*
Jun Araya, *Associate Professor*
Keisuke Saito, *Assistant Professor*
Hiromichi Hara, *Assistant Professor*

General Summary

We address clinical and basic research concerning COPD, bronchial asthma, pulmonary infection, pulmonary fibrosis, and lung cancer. Basic research should resolve clinical problems and clinical research should construct novel treatments. We investigated COPD and IPF pathogenesis concerning cellular senescence and autophagy, and published several reports. We also collaborate with National Cancer Research center concerning EGFR mutation detection and exosome research in the field of lung cancer treatment. We will further extend our research to develop novel treatments against devastating lung diseases.

Research Activities

Cellular senescence and autophagy in chronic obstructive pulmonary disease (COPD)
Chronic obstructive pulmonary disease (COPD) is caused by the noxious effects of tobacco smoke, which leads to airway epithelial cell injury and the induction of phenotypic changes such as squamous metaplasia and cellular senescence, which are assumed to be part of the adaptive response to toxic components such as reactive oxygen species (ROS). Cigarette smoke-induced accelerated cell senescence has been widely implicated in COPD pathogenesis. The accumulation of damaged proteins and organelles are typical manifestations of cellular senescence, indicating the involvement of autophagy, a bulk degradation pathway for cellular components, in the regulation of cellular senescence in COPD. We found that cigarette smoke extract (CSE) treatment of human bronchial epithelial cells (HBEC) transiently induced activation of autophagy, which was associated with accelerated cellular senescence and concomitant accumulations of p62 and ubiquitinated proteins. Autophagy induction in response to CSE was significantly decreased in HBEC from COPD patients, and both p62 and ubiquitinated protein levels were increased in lung homogenates from COPD patients, suggesting the involvement of insufficient p62-mediated selective autophagic clearance of ubiquitinated proteins in accelerated cellular senescence in COPD pathogenesis (Fujii S, et al, Oncoimmunology 1:630-641, 2012).
Mitochondria are dynamic organelles that are essential for cellular metabolic functions, which continuously change their shape through fission and fusion. The proper regulation of mitochondrial dynamics is crucial for the maintenance of functional mitochondria and hence disruption of dynamics induces excessive reactive oxygen species (ROS) production, resulting in apoptosis and cellular senescence. Accelerated cellular senescence is

implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD). Accordingly, we investigated the involvement of mitochondrial dynamics in cigarette smoke extract (CSE)-induced cellular senescence in human bronchial epithelial cells (HBEC). CSE induced mitochondrial fragmentation and mitochondrial oxidative stress, which were responsible for acceleration of cellular senescence in HBEC. Both mitochondrial fragmentation and mitochondrial oxidative stress induced by CSE treatment were inhibited in the presence of NAC or Mito TEMPO. Mitochondrial fragmentation induced by knockdown of fusion proteins also increased mitochondrial ROS production and percentages of senescent cells. CSE-induced mitochondrial fragmentation is involved in cellular senescence through the mechanism of mitochondrial ROS production. Hence, disruption of mitochondrial dynamics may be a part of the pathogenic sequence of COPD development (Hara H, *et al*: *Am J Physiol Lung Cell Mol Physiol* 305: L737-746, 2013).
We also investigated the role of exosome. Exosome is one of extracellular vesicles which have important roles in cell to cell communications. MicroRNA have potential roles in cellular homeostasis and pathophysiology of various diseases. We found that mir201 suppresses ATG7 and autophagy, which leads to fibrogenesis in airway walls of COPD (Fujita Y *et al. J Extracellular Vesicles* 2015).

Cellular senescence and autophagy in idiopathic pulmonary fibrosis
Aberrant re-epithelialization with bronchial epithelial cells is a prominent pathologic finding in idiopathic pulmonary fibrosis (IPF) and is implicated in abnormal epithelial-mesenchymal interactions. Recent studies show senescence as a risk factor for development of IPF. We have produced evidence that IPF lungs show enhanced senescence with a concomitant increase of SIRT6 expression in epithelial cells, including aberrantly re-epithelialized bronchial cells. TGF-β induces senescence by increasing p21 expression and also induces SIRT6 expression, and artificial overexpression of SIRT6 efficiently inhibits TGF-β induced senescence via proteasomal degradation of p21 in HBEC. IL-1β secretion from TGF-β-induced senescent HBEC is responsible for myofibroblast differentiation in fibroblasts. These findings shed light on the accelerated epithelial senescence in IPF pathogenesis with a possible regulatory role for SIRT6 (Minagawa S, et al. Am J Physiol Lung Cell Mol Physiol. 300: L391-401, 2011).
Accelerated epithelial cell senescence accompanied by excessive myofibroblast proliferation has been implicated in the pathogenesis of IPF. Autophagy plays an important regulatory role in cellular senescence and differentiation. Autophagy has been shown to prevent cellular senescence caused by tunicamycin-induced ER stress in human bronchial epithelial cells (HBEC). Conversely, autophagy inhibition was sufficient to induce myofibroblast differentiation in lung fibroblasts. We also demonstrated that metaplastic epithelial cells and fibroblasts in fibroblastic foci (FF) expressed both ubiquitinated proteins and p62 in IPF. Cellular senescence as measured by p21 expression and senescence associated SA-β-Gal staining was observed in metaplastic epithelial cells covering fibrosing lesions. AECII in relatively normal areas of IPF exhibited ubiquitin staining, however a concomitant increase of LC3, indicating autophagy activation, may explain why p21 expression was not observed in those cells. These findings suggest that insufficient autophagy is a potent underlying pathology of both accelerated cellular senescence and myofibroblast

differentiation in a cell-type specific manner and is a promising clue for understanding the molecular mechanisms of IPF (Araya J, Am J Physiol Lung Cell Mol Physiol 304: L56-69, 2013).
Insufficient mitophagy, which is specific autophagy for mitochondria, leads to the accumulation of injured mitochondria, which produce excessive reactive oxygen species (ROS). Excessive ROS activate PDGFR, which results in augmentation of AKT-mTOR pathway. Activation of mTOR induces fibroblatst to omyofibroblats differentiation, and also inhibits autophagy (Kobayashi K et al. J Immunol 197: 504-16, 2016).

Etiologies of in community-acquired pneumonia in adults by real-time polymerase chain reaction

Recently multiplex polymerase chain reaction (PCR) has been applied to detect effectively both respiratory bacteria and viruses. To evaluate etiologies in community-acquired pneumonia in adults, a rapid reliable process based on real-time PCR for respiratory samples was used. We analyzed respiratory tract samples by comprehensive real-time PCR. We prospectively studied 92 patients with COPD using nasopharyngeal swab and sputum samples. Streptococcus pneumoniae was most frequently identified, followed by Haemophilus influenzae and Mycoplasma pneumoniae. PCR also identified viral pathogens. Real-time PCR of nasopharygeal and sputum samples could better identify bacterial and viral pathogens in community-acquired pneumonia than conventional methods (Yoshii Y, et al. Infec Dis 48: 782-8, 2016).

Extracellular Vesicles in Chronic Obstructive Pulmonary Disease

Although several mechanisms of COPD pathogenesis have been verified, the precise mechanism remains unknown. Extracellular vesicles (EVs), including exosomes, microvesicles, and apoptotic bodies, are released from almost all cell types and are recognized as novel cell-cell communication tools. They have been shown to carry and transfer a wide variety of molecules, such as microRNAs, messenger RNAs, and proteins, which are involved in physiological functions and the pathology of various diseases. We address EV-mediated COPD pathogenesis and also investigate the usefulness of EVs as biomarkers (Kadota T, et al. Int J Mol Sci, 2016).

Publications

Inoue A, Yoshida K, Morita S, Imamura F, Seto T, Okamoto I, Nakagawa K, Yamamoto N, Muto S, Fukuoka M. Characteristics and overall survival of EGFR mutation-positive non-small cell lung cancer treated with EGFR tyrosine kinase inhibitors: a retrospective analysis for 1660 Japanese patients. *Jpn J Clin Oncol.* 2016 May; **46:** 462-7. Epub 2016 Mar 13.

Kobayashi K, Araya J, Minagawa S, Hara H, Saito N, Kadota T, Sato N, Yoshida M, Tsubouchi K, Kurita Y, Ito S, Fujita Y, Takasaka N, Utsumi H, Yanagisawa H, Hashimoto M, Wakui H, Kojima J, Shimizu K, Numata T, Kawaishi M, Kaneko Y, Asano H, Yamashita M, Odaka M, Morikawa T, Nakayama K, Kuwano K. Involvement of PARK2-Mediated Mitophagy in Idiopathic Pulmonary Fibrosis Pathogenesis. *J Immunol.* 2016 Jul 15; **197:** 504-16. Epub 2016 Jun 8.

Hosoda C, Baba T, Hagiwara E, Ito H, Matsuo N, Kitamura H, Iwasawa T, Okudela K, Takemura T, Ogura T. Clinical features of usual interstitial pneumonia with anti-neutrophil cytoplasmic antibody in comparison with idiopathic pulmonary fibrosis. *Respirology.* 2016 Jul; **21**: 920-6. Epub 2016 Mar 19.

Iwata T, Yoshino I, Yoshida S, Ikeda N, Tsuboi M, Asato Y, Katakami N, Sakamoto K,

Yamashita Y, Okami J, Mitsudomi T, Yamashita M, Yokouchi H, Okubo K, Okada M, Takenoyama M, Chida M, Tomii K, Matsuura M, Azuma A, Iwasawa T, Kuwano K, Sakai S, Hiroshima K, Fukuoka J, Yoshimura K, Tada H, Nakagawa K, Nakanishi Y. A phase II trial evaluating the efficacy and safety of perioperative pirfenidone for prevention of acute exacerbation of idiopathic pulmonary fibrosis in lung cancer patients undergoing pulmonary resection: West Japan Oncology Group 6711 L (PEOPLE Study). *Respir Res.* 2016 Jul 22; **17:** 90.

Yamakawa H, Hagiwara E, Kitamura H, Yamanaka Y, Ikeda S, Sekine A, Baba T, Iso S, Okudela K, Iwasawa T, Takemura T, Kuwano K, Ogura T. Clinical Features of Idiopathic Interstitial Pneumonia with Systemic Sclerosis-Related Autoantibody in Comparison with Interstitial Pneumonia with Systemic Sclerosis. *PLoS One.* 2016 Aug 26; **11:** e0161908.

Sato N, Takasaka N, Yoshida M, Tsubouchi K, Minagawa S, Araya J, Saito N, Fujita Y, Kurita Y, Kobayashi K, Ito S, Hara H, Kadota T, Yanagisawa H, Hashimoto M, Utsumi H, Wakui H, Kojima J, Numata T, Kaneko Y, Odaka M, Morikawa T, Nakayama K, Kohrogi H, Kuwano K. Metformin attenuates lung fibrosis development via NOX4 suppression. *Respir Res.* 2016 Aug 30; **17:** 107.

Saito Z, Kaneko Y, Kinoshita A, Kurita Y, Odashima K, Horikiri T, Yoshii Y, Seki A, Seki Y, Takeda H, Kuwano K. Effectiveness of hepatoprotective drugs for anti-tuberculosis drug-induced hepatotoxicity: a retrospective analysis. *BMC Infect Dis.* 2016 Nov 11; **16:** 668.

Yoshii Y, Shimizu K, Morozumi M, Chiba N, Ubukata K, Uruga H, Hanada S, Wakui H, Ito S, Takasaka N, Minagawa S, Kojima J, Numata T, Hara H, Kawaishi M, Saito K, Araya J, Kaneko Y, Nakayama K, Kishi K, Kuwano K. Identification of pathogens by comprehensive real-time PCR versus conventional methods in community-acquired pneumonia in Japanese adults. *Infect Dis (Lond).* 2016 Nov-Dec; **48:** 782-8. Epub 2016 Jun 22.

Moroishi T, Hayashi T, Pan WW, Fujita Y, Holt MV, Qin J, Carson DA, Guan KL. The Hippo Pathway Kinases LATS1/2 Suppress Cancer Immunity. *Cell.* 2016 Dec 1; **167:** 1525-39.

Kanda S, Goto K, Shiraishi H, Kubo E, Tanaka A, Utsumi H, Sunami K, Kitazono S, Mizugaki H, Horinouchi H, Fujiwara Y, Nokihara H, Yamamoto N, Hozumi H, Tamura T. Safety and efficacy of nivolumab and standard chemotherapy drug combination in patients with advanced non-small-cell lung cancer: a four arms phase Ib study. *Ann Oncol.* 2016 Dec; **27:** 2242-50. Epub 2016 Oct 20.

Reviews and Books

Fujita Y, Yoshioka Y, Ochiya T. Extracellular vesicle transfer of cancer pathogenic components. *Cancer Sci.* 2016 Apr; **107:** 385-90. Epub 2016 Mar 18.

Kosaka N, Yoshioka Y, Fujita Y, Ochiya T. Versatile roles of extracellular vesicles in cancer. *J Clin Invest.* 2016 Apr 1; **126:** 1163-72. Epub 2016 Mar 14.

Kadota T, Fujita Y, Yoshioka Y, Araya J, Kuwano K, Ochiya T. Extracellular Vesicles in Chronic Obstructive Pulmonary Disease. *Int J Mol Sci.* 2016 Oct 27; **17:** E1801.

Kuwano K, Araya J, Hara H, Minagawa S, Takasaka N, Ito S, Kobayashi K, Nakayama K. Cellular senescence and autophagy in the pathogenesis of chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). *Respir Investig.* 2016 Nov; **54:** 397-406. Epub 2016 May 24.

Kuwano K, Araya J, Hara H, Minagawa S, Takasaka N, Ito S, Nakayama K. Pathogenesis of COPD 4-Cell death, senescence, and autophagy: Is there a possibility of developing new drugs from the standpoint of this pathogenic mechanism? In: Chronic obstructive pulmonary disease: A Systemic Inflammatory Disease. Singapore: Springer; 2016. p. 95-111.

Kuwano K, Araya J, Hara H. Epidemiology and risk factors of idiopathic pulmonary fibrosis (IPF). In: Idiopathic Pulmonary Fibrosis: Advances in Diagnostic Tools and Disease Management. Tokyo: Springer; 2016. p. 11-25.

Department of Internal Medicine
Division of General Medicine

Iwao Ohno, *Professor*
Hiroshi Yoshida, *Professor*
Yasuhiko Miura, *Associate Professor*
Jun Hiramoto, *Associate Professor*
Hideo Okonogi, *Assistant Professor*
Masami Nemoto, *Professor*
Joji Otsuki, *Associate Professor*
Nobuyuki Furutani, *Associate Professor*
Tatsuhiro Joki, *Associate Professor*

General Summary

1. Management of a database of our medical examinations and treatments.
2. Planning a post-graduate training program to acquire skills for the general practice required in the community.

Research Activities

Division of General Medicine, The Jikei University Hospital
We are attempting to compile a database of our medical examinations and treatments during primary care in outpatient units. The data and information of every outpatient are collected from forms of our own design after being filled out by physicians. The data and information include reason for visiting, symptoms and complains, whether the patient had consulted other physicians, the primary diagnosis, examinations, and care. The frequent consultation reason was abdominal pain, cough and pyrexia. Then, frequent initial diagnosis was upper respiratory tract infection, infectious gastroenteritis and headache. The data we compile, especially from initial visits, are expected to be useful for analyzing trends in primary care at large general hospitals.
Recently, there is a strong desire of change in medical education, from hospital-based specialty care to first-line health care, including community health care. Thus, we are now planning the post-graduate and continuing professional development of physicians to acquire skills for the general practice required in the community.

Division of General Medicine, The Jikei University Katsushika Medical Center
We experienced three valuable cases. We discovered that a parasite was the cause of the disease in a case of eosinophillic meningitis. In a case of beriberi with leg edema, pleural effusion and anemia, we clarified that vitamin B1 deficiency was observed and should be taken care of at present. We presented the method of diagnosis and the therapy in a case report of fibromyalgia in Japan.
We also started a basic research about gas biomarkers from skin and expiration from lung. We are going to examine about the metabolic and inflammatory changes in the patients with obesity, collagen disease and vitamin B1 deficiency.

Division of General Medicine, The Jikei University Daisan Hospital
1. Study of polymyalgia rheumatic (PMR)

We found that bursitis on US is detected only 50% patients. We can cure about 50% cases, and the other cases need to treat continuously.
2. Study about DNAR
We discussed about DNAR(Do not attempt resuscitation) and POLST(Physician Orders for Life-sustaining Treatment) in university hospital. Almost all doctors and nurses know DNAR, but about 60% were confused in actual cases. Only 2% know POLST.

Division of General Medicine, The Jikei University Kashiwa Hospital
Our research in The Jikei University Kashiwa Hospital consists of 3 parts. The first is to develop inter-professional work in Kashiwa area. We conducted the open seminar related to general medicine and clinical ethics 3-4 times in a year.
The second part is to develop educational tasks for teaching medical students and junior physicians. We developed the education system using WEB (called e-Portfolio) and now running.
The third part is concerning the management of Hospital Ethics Committee and Clinical Ethics Consultation in The Jikei University Kashiwa Hospital. There were 9 consultations last year and now these cases were under reconsideration for presentation and publication. We are running a research concerning POLST Japanese version in Japanese hospital setting.

Other activities
1. Fibromyalgia
We constructed the chronic water abuse and filled bag muscle theory as new pathophysiologic theorem of fibromyalgia. We started to plan basic and clinical researches and new series of treatment for various fibromyalgia patients.
2. Development of e-Portfolio for junior resident education
We are developing e-Portfolio system continuously to enforce efficacy and effectiveness. The systems are on the stage of stable and new development among Kashiwa, Katsushika and Daisan hospitals.
3. Theoretic construction of the General Medical
We constructed a theorem of General Medicine as a new academic discipline. That is mainly aimed to elucidate disease structures of unsystemized disease structures and to construct integrated symptom based diagnosis.

Publications

Tsutsui K, Nemoto M. A case of beriberi with leg edema, pleural effusion, and anemia. *Annals of Clinical Case Reports.* 2016; **1:** 1067.

Tsutsui K, Nemoto M. A case report of fibromyalgia. *Annals of Clinical Case Reports.* Epub 2016 Nov 3.

Department of Psychiatry

Kazuhiko Nakayama, *Chairman and Professor*
Kei Nakamura, *Professor*
Hironari Sue, *Professor*
Wataru Yamadera, *Associate Professor*
Kazuya Ono, *Associate Professor*
Ayumi Tateno, *Associate Professor*
Tatsuhiko Itoh, *Assistant Professor*
Masanori Kawakami, *Assistant Professor*
Fumitoshi Kodaka, *Assistant Professor*
Hiroshi Itoh, *Professor*
Hisatsugu Miyata, *Professor*
Kazutaka Nukariya, *Associate Professor*
Motohiro Ozone, *Associate Professor*
Rieko Shioji, *Associate Professor*
Minako Koga, *Associate Professor*
Satoshi Kawamura, *Assistant Professor*
Shunichiro Shinagawa, *Assistant Professor*

General Summary

Our research activities cover a wide range of topics: disorders at the psychologic and biologic levels, from childhood and adolescence, through adulthood, to the senile period. Sociologic, psychologic, physiologic, and biochemical methods were used.

Research Activities

Psychopathology, psychotherapy and child study group
We have been studying in the fileds of psychotherapy, psychopathological and, child psychiatry. In our study on the relationship between developmental disorder and personality disorder, we published a hybrid hypothesis, a hypothesis that bridges the relationship between developmental disability and personalities. We have been studying the understanding and treatment strategies of various diseases; A Study on the Relationship between Religion and Psychotherapy (Ono) Investigation of attention function comparing general psychiatric disorder and developmental disorder (Okino), Long-term prognostic study of borderline personality disorder (Onoda Onoda), Family to ASD long-term withdrawal case Therapeutic Approach (Sugihara), etc. In child psychiatric research, we studied the application of dialectic behavioral therapy to developmental disorders since adolescence, prepared a manual and prepared for enforcement. It was. In addition, in the scientific research conducted by the Ministry of Health, Labor and Welfare, "Survey on Child psychiatrists' involvement with support for intellectual and developmental disabled people in behavioral disorder" was the second year. In this research, a questionnaire survey was conducted for member doctors of the Japan Child and Adolescent Psychiatric Association for the purpose of clarifying to what extent the child psychiatrist has involvement and what kind of difficulties are involved in the disability welfare field. As a result of this survey, nearly half of the child psychiatrists in this field covered, but they also felt various difficulties, and it is necessary to expand specialized training, need for education of staff, enhancement of facilities facilities, medical cooperation These four points of securing the system were considered important in the future.

Morita therapy group
In cooperation with psychotherapists of other schools such as cognitive-behavioral ther-

apy and psychoanalysis, we have been developing programs and materials to train young psychiatrists in order to master the basic techniques of the clinical interview. We continued the following studies this year: practical research towards OCD with Autistic Spectrum Disorder, practical research towards the application of Morita therapy to the patients in adolescence and the patients with「Hikikomori (withdrawal)」, the psychopathology of social anxiety disorders, factors in the recovery of patients with depression through inpatient Morita therapy, and the application of Morita therapy to the field of palliative medicine.

Psychopharmacology group

In basic research, we performed the following studies in rodents: 1) effect of novel psychotropic on monoamine neurotransmission using microdialysis and radioimmunoassay technique, 2) formation mechanism of drug addiction, 3) neural basis of addiction-related impulsivity, and 4) development of novel anti-craving agent (the final 3 subjects were performed in collaboration with the NTT Communication Science Laboratories and the Department of Psychology, Senshu University). In clinical research, we performed the following studies in humans: 1) the developmental and the psychological and social predictors of recovery in patients with schizophrenia study, 2) regulation of the salience network with antipsychotic agents via dopamine D2/3 receptors, 3) qualitative research on adherence in patients with schizophrenia, 4) the effect of modified electroconvulsive therapy on regulatory factors for gene expression, and 5) symptomatology in menstruation-related mental disorders, atypical psychosis, and acute psychosis. Integration between basic and clinical research is a fundamental concept of the Psychopharmacology group.

Psychophysiology group

Studies examined: 1) changes in sleep structures by cognitive behavioral therapy for insomnia using the cyclic alternating pattern method (CAP), 2) the empirical research regarding the efficacy of the group cognitive behavioral therapy for primary insomnia and depression, 3) The clinical research using MSLT in terms of hypersomnias of central origin, 4) The investigations of bio-markers of fatigue for obstructive sleep apnea syndrome.

Psychogeriatric group

We are currently undertaking a several researches investigating neural basis of neuropsychiatric symptoms and social functions in patients with neurodegenerative disorders and other psychiatric disorder among elderly people using neuropsychological testing, neuroimaging methods such as brain MRI and SPECT, and genetic testing. One study focused on compensation mechanism of anosognosia in Alzheimer's disease (AD), and investigated neural correlates of anosognosia in AD. As a result, we revealed that anosognosia in AD could be correlated with executive dysfunction as well as functional compensation through semantic memory system. Another study focused on effect of DNA methylation on the pathogenesis of AD. Our results suggest that DNA methylation in the NCAPH2/LMF2 promoter region can be a useful biomarker for the diagnosis of AD and amnesic mild cognitive impairment (aMCI), which is associated with hippocampal atrophy through apoptosis. We are going to investigate further impacts of these mechanisms on

patients' behavioral and psychological symptoms of dementia.

General hospital psychiatry group
In a study of interventional therapy based on cognitive-behavioral therapy aimed at preventing recurrences of depression, a computer system and sleep evaluation methods were introduced in addition to a previous evaluation system for more effective presentations and for more precise estimation, respectively. Furthermore, an investigation of new indications for this intervention for atypical depression, bipolar depression, and insomnia was performed. Another study investigated the issues associated with mental care services for patients with digestive tract cancers.

Clinical electroencephalography group
We tried interpretation based on Neojacksonism(Ey H) about the cases that presented the psychotic symptoms associated with epilepsy. Furthermore we reported changes in serum concentrations of AEDs (especially new-antiepileptic drugs) during pregnancy of epileptic patients. A study was performed to prevent the recurrence of depression in patients with epilepsy. We examined the safety and efficacy of psychotropic drugs in several forms of psychosis associated with epilepsy.

Clinical psychology group
We have continued to discuss and study psychotherapeutic processes and the treatment techniques of cognitive behavior therapy, Morita therapy, relief care, psycho-oncology, and social skill training. We have also examined the characteristics of developmental disorders and higher brain dysfunctions through psychological assessments. We invited Dr. Toshikazu Shinotake at a clinical conference and study 'Reading data from psychological tests for developmental disorders-with a focus on WAIS-III', and how to assess them in clinical scenes. Furthermore, we trained graduate students of a clinical psychological course.

Publications

Chung JK, Nakajima S, Shinagawa S, Plitman E, Iwata Y, Gerretsen P, Chakravarty MM, Caravaggio F, Pollock BG, Graff-Guerrero A. Benzodiazepine Use Attenuates Cortical Beta-Amyloid and is Not Associated with Progressive Cognitive Decline in Non-Demented Elderly Adults: A Pilot Study Using F18-Florbetapir Positron Emission Tomography. *Am J Geriatr Psychiatry.* 2016; **24:** 1028-39.

Fujino J, Fujimoto S, Kodaka F, Camerer CF, Kawada R, Tsurumi K, Tei S, Isobe M, Miyata J, Sugihara G, Yamada M, Fukuyama H, Murai T, Takahashi H. Neural mechanisms and personality correlates of the sunk cost effect. *Sci Rep.* 2016; **6:** 33171.

Inamura K, Sinagawa S, Nagata T, Tagai K, Nukariya K, Nakayama K. Executive Dysfunctions Correlated with 2-Year Treatment Response in Patients with Late-Life Undifferentiated Somatoform Disorders. *Psychosomatics.* 2016; **57:** 378-89.

Nakano T, Kodaka F, Tsuneoka H. Differences in Neuroticism Between Patients with Glaucoma Who Have Discontinued Visits to Ophthalmologists and Those Who Make Regular Visits: Implications for Adherence to Topical Glaucoma Medications. *Ophthalmol Ther.* 2016; **5:** 207-14.

Kobayashi N, Shinagawa S, Nagata T, Shimada K, Shibata N, Ohnuma T, Kasanuki K, Arai H, Yamada H, Nakayama K, Kondo K. Usefulness of DNA Methylation Levels in COASY and SPINT1 Gene Promoter Regions as Biomarkers in Diagnosis of Alzheimer's Disease and Amnestic Mild Cognitive Impairment. *PLoS One.* 2016 Dec 19; **11:** e0168816.

Aoki R, Kobayashi N, Suzuki G, Kuratsune H,

Shimada K, Oka N, Takahashi M, Yamadera W, Iwashita M, Tokuno S, Nibuya M, Tanichi M, Mukai Y, Mitani K, Kondo K, Ito H, Nakayama K. Human herpesvirus 6 and 7 are biomarkers for fatigue, which distinguish between physiological fatigue and pathological fatigue. *Biochem Biophys Res Commun.* 2016; **478:** 424–30.

Shinagawa S, Honda K, Kashibayashi T, Shigenobu K, Nakayama K, Ikeda M. Classifying eating-related problems among institutionalized peaple with dementia. *Psychiatry Clin Neurosci.* 2016; **70:** 175–81.

Shinagawa S, Kobayashi N, Nagata T, Kusaka A, Yamada H, Kondo K, Nakayama K. DNA methylation in the NCAPH2/LMF2 promoter region is associated with hippocampal atrophy in Alzheimer's disease and amnesic mild cognitive impairment patients. *Neurosci Lett.* 2016; **629:** 33–7.

Yamadera W, Morita M. Psychophysiological evaluations of clinical efficacy in outpatients Morita therapy for psychophysiological insomnia. *J Sleep Disord Ther.* 2016; **5:** 235.

Yamadera W, Morita M, Sakamoto S, Kuroda A, Itoh H, Nakayama K. Comparison of escitalopram alone and combined with zolpidem in treating major depression and related sleep impairments. *Sleep Biol Rhythms.* 2016; **16:** 303–8.

Takeshita Y, Shibata N, Kasanuki K, Nagata T, Shinagawa S, Kobayashi N, Ohnuma T, Suzuki A, Kawai E, Takayama T, Nishioka K, Motoi Y, Hattori N, Nakayama K, Yamada H, Arai H. Genetic association between RAGE polymorphisms and Alzheimer's disease and Lewy body dementias in a Japanese cohort: a case-control study. *Int J Geriatr Psychiatry*. Epub 2016 Oct 4.

Department of Pediatrics

Hiyoruki Ida, *Professor*
Mitsuyushi Urashima, *Professor*
Ichiro Miyata, *Associate Professor*
Yoshihiro Saito, *Associate Professor*
Hiroshi Kobayashi, *Associate Professor*
Hiroshi Tachimoto, *Assistant Professor*
Masahisa Kobayashi, *Assistant Professor*
Toya Ohashi, *Professor*
Yasuyuki Wada, *Professor*
Toshio Katsunuma, *Associate Professor*
Yoko Kato, *Associate Professor*
Masako Fujiwara, *Assistant Professor*
Masaharu Akiyama, *Assistant Professor*
Takashi Urashima, *Assistant Professor*

General Summary

We have 10 subspecialty research groups consisting of the Inherited Metabolic Disease group, the Endocrinology group, the Neurology group, the Hematology and Oncology group, the Infectious Diseases and Immunologic Disorders group, the Nephrology group, the Cardiology group, the Allergy group, the Neonatology group, and the Pediatric Psychiatry group. The final aim of each subspecialty groups is supplying practical benefits to patients and their families through basic and translational research and clinical study.

Research Activities

Inherited metabolic disease group
The phenotype of peripheral neuropathy of murine model of Fabry disease is hypoesthesia. AAV vector expressing alfa-galactosidase A (GLA) was administered into intrathecal space of model mice. GLA activity in dorsal root ganglion was elevated and hypoesthesia was improved.
We previously shown that hematopoietic stem cell targeted gene therapy using lentiviral vector was effective for mucopolysaccharidosis type II mice. This year, to test human hematopoietic stem cell targeted lentiviral gene therapy is also effective, we generated immune deficient mucopolysaccharidosis type II mice which human cell can be transplantable using gene editing technology. The enzyme activity was decreased in organ in 3 strains. Next, we will treat the mice by human hematopoietic stem cell targeted gene therapy using lentiviral vector for future clinical trial.

Neurology group
We are conducting a research on Dravet syndrome by using disease-specific induced pluripotent stem cells and knockout rats. The aims include to elucidate the developmental molecular and cellular pathology and to explore the possibility of cell therapy. In 2006, to anatomically identify epileptogenic brain area/neural circuits in the disease rats, we newly started an experiment to depict hyperexcitable regions by using a manganese-enhanced magnetic resonance imaging technique. This analysis would be helpful to determine regions to be targeted in cell therapy and to assess efficacy of the treatment. In another study, we performed a clinical survey of 70 cases of West syndrome and determined the efficacy and safety of intravenous immunoglobulin therapy in this disease.

Nephrology group
We conducted a nationwide survey for pediatric end-stage kidney disease (ESKD) and examined the level of estimated glomerular filtration rate (eGFR) at the start of renal replacement therapy (RRT). We found a possible association between baseline eGFR and subsequent survival outcome.
We also identified possible risk factors for prolonged hematuria after methylprednisolone pulse therapy combined with tonsillectomy in childhood-onset IgA patients, and found that severe crescents formation was associated with prolonged hematuria.

Infectious diseases and Immunologic Disorders group
We studied human herpes virus-6 (HHV-6) reactivation in the central nerve system. We reported that IL-1β and basic fibroblast growth factor (bFGF) are important factors for proliferation of HHV-6 in astrocyte cell line and are elevated in the cerebrospinal fluid of patients with HHV-6 encephalitis. Our results indicate that IL-1β and bFGF play a key role in the onset of HHV-6 encephalitis.
We investigated early diagnosis and treatment for primary immunodeficiency diseases at Department of Human Genetics in National Research Institute for Child Health and Development. We have prepared for newborn screening to detect treatable severe primary immunodeficiency diseases threatening to life or long-term health, before they become symptomatic.

Hematology and Oncology group
We have performed several clinical studies for hematologic malignancies as a member of Japan Child Cancer Study Group (JCCG) and Tokyo Children's Cancer Study Group (TCCSG) to explore novel therapy and diagnostic tool. We investigated the management of chronic idiopathic thrombocytopenic purpura in children by questionaires to hospitals. Genomic analysis of clonal origin of Langerhans cell histiocytosis (LCH) following acute lymphoblastic leukemia (ALL) was performed. We demonstrated that homozygous deletion of *CDKN2A* at 9p21 of 72 kb was detected in the specimen of LCH following ALL by SNP assay. In addition, *NRAS* c.G34A mutation was found in the specimen.
We studied the molecular effects of GNAS-cAMP-dependent protein kinase A-sonic hedgehog (SHH) coupling on progression of sonic hedgehog-driven medulloblastoma.

Cardiology group
We evaluated right ventricular remodeling using right ventricular pressure overload mouse, right ventricular fibrosis in response to pressure overload in rats using two-dimensional speckle tracking echocardiography and the mechanism of angiogenesis using the model rat with aorto-pulmonary collateral artery. Moreover, we made model rat with pulmonary hypertension caused by left heart disease and evaluated intrapulmonary venous arterialization. We have performed following studies; technical investigation of intervention catheterization, cardiac function, hepatic fibrosis, and protein losing enteropathy after Fontan operation, and safety management of congenital heart disease at pediatric intensive care unit.

Allergy group

The main subjects of our research are as follows: 1) the role of eosinophil, mast cells and epithelial cells in the pathology of allergic diseases, 2) pediatric asthma, 3) food allergy, 4) atopic dermatitis, 5) treatments for allergic diseases, and 6) prevention of allergic diseases. We have been organized and performed following multicenter randomized trials. Recently, olfactory function in children with rhinitis has been investigated, which is the first study in the world as far as we know.

Endocrinology group

We investigated whether thyroid dysfunction affects pubertal onset in female mice via gonadotropin-inhibitory hormone (GnIH). As a result, hypothyroidism showed delayed puberty onset with increased GnIH expression and reduced pituitary gonadal activity. This finding indicates a novel function of GnIH to mediate hypothalamic-pituitary-thyroid axis (HPT) - hypothalamic-pituitary-gonadal axis (HPG) interactions that contribute to proper pubertal development. Moreover, we have performed intervention studies to prevent metabolic syndrome in children by using exercise therapy that was newly developed.

Publications

Akashi K, Mezawa H, Tabata Y, Atsuta J, Tokuda R, Kawada Y, Kitamura T, Murasugi H, Ito H, Tabata M, Shirao K, Sato S, Nishimura H, Fujiwara M, Masuda K, Arakawa H, Adachi Y, Yoshihara S, Fujisawa T, Katsunuma T. Optimal step-down approach for pediatric asthma controlled by salmeterol/fluticasone: A randomized, controlled trial (OSCAR study). *Allergol Int.* 2016; **65:** 306-11.

Hirano D, Ishikura K (National Center for Child Health and Development), Uemura O (Aichi Children's Health and Medical Center), Ito S (Yokohama City University), Wada N (Shizuoka Children's Hospital), Hattori M (Tokyo Women's Medical University), Ohashi Y (Chuo University), Hamasaki Y (Toho University Faculty of Medicine), Tanaka R (Hyogo Prefectural Kobe Children's Hospital), Nakanishi K (Wakayama Medical University), Kaneko T (Tokyo Metropolitan Children's Medical Center), Honda M (Tokyo Metropolitan Children's Medical Center); Pediatric CKD Study Group in Japan in conjunction with the Committee of Measures for Pediatric CKD of the Japanese Society of Pediatric Nephrology. Association between low birth weight and childhood-onset chronic kidney disease in Japan: a combined analysis of a nationwide survey for paediatric chronic kidney disease and the National Vital Statistics Report. *Nephrol Dial Transplant*. 2016; **31:** 1895-900.

Horimukai K, Morita K, Narita M, Kondo M, Kabashima S, Inoue E, Sasaki T, Niizeki H, Saito H, Matsumoto K, Ohya Y. Transepidermal water loss measurement during infancy can predict the subsequent development of atopic dermatitis regardless of filaggrin mutations. *Allergol Int.* 2016; **65:** 103-8.

Ida H, Tanaka A, Matsubayashi T, Murayama K, Hongo T, Hak-Myung Lee, Mellgard B. A multicenter, open-label extension study of velaglucerase alfa in Japanese patients Gaucher disease: Results after a cumulative treatment period of 24 months. *Blood Cells Mol and Dis.* 2016; **59:** 140-7.

Inoue T, Akashi K, Watanabe M, Ikeda Y, Ashizuka S, Motoki T, Suzuki R, Sagara N, Yanagida N, Sato S, Ebisawa M, Ohta S, Ono J, Izuhara K, Katsunuma T. Periostin as a biomarker for the diagnosis of pediatric asthma. *Pediatr Allergy Immunol.* 2016; **27:** 521-6.

Kato M, Seki M, Yoshida K, Sato Y, Oyama R, Arakawa Y, Kishimoto H, Taki T, Akiyama M, Shiraishi Y, Chiba K, Tanaka H, Mitsuki N, Kajiwara M, Mizutani S, Sanada M, Miyano S, Ogawa S, Koh K, Takita J. Genomic analysis of clonal origin of Langerhans cell histiocytosis following acute lymphoblastic leukaemia. *Br J Haematol.* 2016; **175:** 169-72.

Katsunuma T, Adachi Y, Miura K, Teramoto T, Fujisawa T, Ohya Y; Working Group for the Disaster, Japanese Society of Pediatric Allergy and Clinical Immunology. Care of children with allergic diseases following major disasters. *Pediatr Allergy Immunol.* 2016; **27:** 425.

Matsuura R, Hamano S, Hirata Y, Oba A, Suzuki K, Kikuchi K. Intravenous immunoglobu-

lin therapy is rarely effective as the initial treatment in West syndrome: a retrospective study of 70 patients. *J Neurol Sci.* 2016; **368:** 140–4.

Ono E, Ariga M, Oshima S, Hayakawa M, Imai M, Ochiai Y, Mochizuki H, Namba N, Ozono K, Miyata I. Three novel mutations of the *MCT8 (SLC16A2)* gene: individual and temporal variations of endocrinological and radiological features. *Clin Pediatr Endocrinol.* 2016; **25:** 23–35.

Sato Y, Kobayashi H, Higuchi T, Shimada Y, Ida H, Ohashi T. TFEB overexpression promotes glycogen clearance of Pompe disease iPSC-derived skeletal muscle. *Mol Ther Methods Clin Dev.* 2016; **3:** 16054.

Tachimoto H, Mezawa H, Segawa T, Akiyama N, Ida H, Urashima M. Improved control of childhood asthma with low-dose, short-term vitamin D supplementation: a randomized, double-blind, placebo-controlled trial. *Allergy.* 2016; **71:** 1001–9.

Tsunogai T, Miyata I, Miwa S, Matsuura R, Takagi K, Nanba H, Takahata N,Tajima T, Wada Y. A novel *NR3C2* mutation in a Japanese patient with the renal form of pseudohypoaldosteronism type 1. *Clin Pediatr Endocrinol.* 2016; **25:** 111–4.

Yokoi K, Yamaoka M, Miyata I, Nonaka Y, Yuza Y, Kawata S, Akiyama M, Yanagisawa T, Ida H. Atypical clinical features of children with central nervous system tumor: Delayed diagnosis and switch in handedness. *Pediatr Int.* 2016; **58:** 923–6.

Yokoi T, Yokoi K, Akiyama K, Higuchi T, Shimada Y, Kobayashi H, Sato T, Ohteki T, Otsu M, Nakauchi H, Ida H, Ohashi T. Non-myeloablative preconditioning with ACK2 (anti-c-kit antibody) is efficient in bone marrow transplantation for murine models of mucopolysaccharidosis type II. *Mol Genet Metab.* 2016; **119:** 232–8.

Department of Dermatology

Hidemi Nakagawa, *Professor*
Akihiko Asahina, *Professor*
Masaaki Kawase, *Associate Professor*
Yoshimasa Nobeyama, *Associate Professor*
Keigo Ito, *Assistant Professor*
Munenari Ito, *Assistant Professor*
Takaoki Ishiji, *Professor*
Arihito Ota, *Associate Professor*
Yoshinori Umezawa, *Associate Professor*
Toshihiro Ito, *Assistant Professor*
Koichi Yanaba, *Assistant Professor*
Yozo Ishiuji, *Assistant Professor*

General Summary

We have organized special clinics for selected skin diseases, including viral diseases, neurofibromatosis type 1, atopic dermatitis, psoriasis, contact dermatitis, and skin cancers. Integrating concentrated clinical efforts and related basic research should provide a significant contribution to excellent clinical practice.

Research Activities

Psoriasis
Various systemic therapies, including oral cyclosporin microemulsion preconcentrate, methotrexate, etretinate, biologics and topical therapies such as vitamin D3, and corticosteroids, have been used, depending on disease severity and the degree of the impairment of patient's quality of life (QOL) in individual patients. Also phototherapy is effective and have been performed in skin-care clinic. We have evaluated patients' QOL and have developed a Japanese version of the Psoriasis Disability Index and the Work Productivity and Activity Impairment questionnaire for psoriasis. In a special psoriasis clinic, we select patient-based treatments to satisfy patients' demands. New biologic agents, including infliximab, adalimumab, ustekinumab, and secukinumab are available and have been used to treat intractable severe psoriasis. Clinical trials have been performed with new biologic agents, including antibodies against IL-23p19 and new topical agents.

Atopic dermatitis
Recently, psychosocial factors have been suggested to influence the exacerbation of atopic dermatitis. Therefore, we are treating patients on the basis of both evidence-based medicine and QOL issues. We try to obtain a precise medical history from each patient and to evaluate the degree of QOL impairment. We are also doing some basic experiments using atopic model mice to reveal the mechanism of pruritus in this disease. Clinical trials of topical phosphodiesterase-4 inhibitor and anti-IL-31 receptor antibody have been performed.

Malignant skin tumors
We have been studying clinical courses, postoperative outcomes, and genomic and expression changes in patients with malignant melanoma, extramammary Paget's disease, squamous cell carcinoma, basal cell carcinoma, cutaneous T-cell lymphomas, and a wide

variety of soft tissue sarcomas including malignant peripheral nerve sheath tumors (MPNSTs). For the accurate diagnosis of pigmented tumors, we always perform dermoscopic examinations and sentinel lymph-node biopsy. For advanced stage cases, we treated the patients with multidisciplinary treatments including immune check point inhibitors, molecular targeted agents, chemotherapy and radiation therapy.

Neurofibromatosis

Because the number of registered patients in our clinic is the largest in Japan, we concentrate on long-term follow-up and improvement of impaired QOL by means of accurate diagnosis and the resection of neurofibromas. The estimated lifetime risk of malignant peripheral nerve sheath tumor (MPNST) in patients with neurofibromatosis 1 is around 10%. We have used the methylation-specific polymerase chain reaction (PCR) and real-time reverse transcriptase (RT)-PCR to analyze the methylation status of tumor suppressor genes and cancer-testis genes in established MPNST cell lines.

Herpes virus infection

1. Herpes simplex virus

Rapid diagnostic procedures by means of immunohistochemical staining with monoclonal antibodies against herpes simplex virus (HSV)-1, HSV-2, and varicella-zoster virus are performed in this clinic. After the diagnosis is confirmed, suppressive therapies with valaciclovir are started to improve the impaired QOL.

2. Herpes zoster and postherpetic neuralgia

Initial treatments for herpes zoster and postherpetic neuralgia (PHN) are performed in this clinic. To prevent PHN, we proactively use tricyclic antidepressants. Posthoc analyses of a subgroup of patients showed that amitriptyline in combination with acyclovir reduced the incidence of PHN. We prescribe pregabalin, tricyclic antidepressants, selective serotonin reuptake inhibitors, opioid analgesics, such as Tramcet® (Grunethal Ltd., Stokenchurch, UK), which contains tramadol hydrochloride and acetominophen.

Human papillomavirus infection

In addition to ordinary cryotherapy, topical vitamin D3, salicylic acid, glutaraldehyde, and monochloro acetic acid have been used to treat viral warts. Contact immunotherapy using squaric acid dibutylester, CO_2 laser, and pulsed dye laser have also been used to treat severe intractable viral warts. Human papillomavirus infection typing with the PCR has regularly been performed.

Contact dermatitis/drug eruption

We have performed patch testing regularly to identify causes of contact dermatitis and drug eruption.

Laser

The Q-switched 694-nm ruby laser is useful for the treatment of nevus of Ota, acquired dermal melanocytosis, and ectopic Mongolian spots. On the other hand, nevus spilus / café-au-lait spots are difficult to treat with the Q-switched ruby laser because they often

recur after 1 to 2 months. The recently introduced 595-nm V-beam laser (long pulsed dye laser) is effective for intractable vascular lesions. The ultra-pulse CO_2 laser can be used to quickly remove lesions of actinic keratosis, seborrheic keratosis, syringoma, and epidermal nevus.

Skin Care Clinic

Narrow-band ultraviolet B (NB-UVB) irradiation is performed for patients with psoriasis, alopecia, atopic dermatitis, prurigo nodularis, vitiligo, or cutaneous T-cell lymphomas. Other special clinics, including those for skin care lessons, therapeutic make-up, acne care, mental care, and *kampo* medicine, are available to patients on demand.

Publications

Uratsuji H, Tada Y, Hau CS, Shibata S, Kamata M, Kawashima T, Asano Y, Sugaya M, Kadono T, Asahina A, Sato S. Monosodium Urate Crystals Induce Functional Expression of P2Y14 Receptor in Human Keratinocytes. *J Invest Dermatol.* 2016; **136:** 1293-6.

Asahina A, Umezawa Y, Yanaba K, Nakagawa H. Serum C-reactive protein levels in Japanese patients with psoriasis and psoriatic arthritis: Long-term differential effects of biologics. *J Dermatol.* 2016; **43:** 779-84.

Asahina A, Etoh T, Igarashi A, Imafuku S, Saeki H, Shibasaki Y, Tomochika Y, Toyoizumi S, Nagaoka M, Ohtsuki M; study investigators. Oral tofacitinib efficacy, safety and tolerability in Japanese patients with moderate to severe plaque psoriasis and psoriatic arthritis: A randomized, double-blind, phase 3 study. *J Dermatol.* 2016; **43:** 869-80.

Asahina A, Torii H, Ohtsuki M, Tokimoto T, Hase H, Tsuchiya T, et al. Safety and efficacy of adalimumab treatment in Japanese patients with psoriasis: Results of SALSA study. *J Dermatol.* 2016; **43:** 1257-66.

Saeki H, Kinoshita Y, Asahina A, Ochiai T, Iijima M. Toxic epidermal necrolysis in Japan in the past 10 years based on data from the relief system of the Pharmaceuticals and Medical Devices Agency. *J Dermatol.* 2017; **44:** e9-10.

Nakayama M, Itoh M, Kikuchi S, Tanito K, Nakagawa H. Thymoma-associated cutaneous graft-versus-host-like disease possibly treated with Narrow-band UVB phototherapy. *Eur J Dermatol.* 2016 Apr 1; **26:** 208-9.

Itoh M, Kawagoe S, Okano HJ, Nakagawa H. Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a patient with lymphedema-distichiasis syndrome (LDS) carrying an insertion-deletion complex mutation in the FOXC2 gene. *Stem Cell Res.* 2016 May; **16:** 611-3.

Itoh M, Kawagoe S, Okano HJ, Nakagawa H. Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a healthy individual: WT-iPSC2. *Stem Cell Res.* 2016 May 11; **17:** 16-8.

Itoh M, Kawagoe S, Okano HJ, Nakagawa H. Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a healthy individual: WT-iPSC4. *Stem Cell Res.* 2016 May 11; **17:** 19-21.

Itoh M, Kawagoe S, Okano HJ, Nakagawa H. Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a healthy individual: WT-iPSC1. *Stem Cell Res.* 2016 May 11; **17:** 22-4.

Itoh M, Kawagoe S, Tamai K, Okano HJ, Nakagawa H. Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a patient with recessive dystrophic epidermolysis bullosa (RDEB) carrying two compound heterozygous mutations in the COL7A1 gene. *Stem Cell Res.* 2016 May17; **17:** 32-5.

Waki Y, Nobeyama Y, Mori A, Tokita M, Itoh M, Nakagawa H. Case ofmetachronous and multifocal extramammary Paget's disease. *J Dermatol.* 2016 Jun; **43:** 723-4.

Yoshikata-Isokawa Y, Itoh M, Nakagawa H. Japanese sporadic case of erythrokeratodermia variabilis caused by the connexin-30.3 (GJB4) mutation: Is Glycine 12 a mutational hotspot in the connexin family? *J Dermatol.* 2016 Jul; **43:** 830-1.

Inokuchi S, Nobeyama Y, Itoh M, Nakagawa H. A case of deep dissecting hematoma: different managements resulting in similar outcomes. *Int J Dermatol.* 2016 Dec; **55:** e628-9.

Fukasawa-Momose M, Itoh M, Ito K, Nobeyama Y, Nakagawa H. Cutaneous apocrine carcinoma on the scalp after cranial irradiation for acute lymphocytic leukaemia. *Eur J Dermatol.* 2016 Dec 1; **26:** 612-3.

Murayama A, Itoh M, Ito K, Tanito K, Ishida K, Nobeyama Y, Nakagawa H. Case of desmoplastic melanoma with lung metastasis maintaining complete response after cessation of nivolumab. *J Dermatol.* 2017 Mar; **44:** e17-8.

Yamaguchi T, Itoh M, Umezawa Y, Asahina A, Hanabusa H, Nakagawa H. Acquired hemophilia

A and fulminant diabetes mellitus possibly caused by adalimumab in a patient with psoriatic arthritis. *J Dermatol.* 2017 Mar; **44:** e3-4.

Sato Y, Nobeyama Y, Omori Y, Nakagawa H. Female case of nevus lipomatosus cutaneous superficialis growing depending on the occurrence site. *J Dermatol.* 2017 Jan; **44:** 107-8.

Kazama M, Umezawa Y, Itoh M, Nobeyama Y, Ito T, Kikuchi S, Yanaba K, Asahina A, Nakagawa H, Ogasawara Y. Successful treatment of ustekinumab in a psoriasis patient with human T-cell leukemia/lymphotropic virus type 1 infection. *J Dermatol*. Epub 2016 Nov 18.

Nobeyama Y, Nakagawa H. Aberrant DNA Methylation in Keratoacanthoma. *PLoS One.* 2016; **11:** e0165370.

Omori Y, Nobeyama Y, Tomita S, Ishida K, Nakagawa H. Case of spindle cell squamous cell carcinoma with dermoscopic findings of diffuse/discrete scales and predominant white color. *J Dermatol.* 2016; **43:** 1239-41.

Nobeyama Y, Nakagawa H. MAGEA3 methylation status is associated with prognosis of malignant peripheral nerve sheath tumor and with neurofibroma type in neurofibromatosis type 1. *J Dermatol Sci.* 2016; **84:** 101-4.

Nobeyama Y, Umezawa Y, Nakagawa H. Less-invasive analysis of DNA methylation using psoriatic scales. *J Dermatol Sci.* 2016; **83:** 70-3.

Miyake S, Nobeyama Y, Baba-Honda H, Nakagawa H. Case of ecthyma gangrenosum in which only methicillin-resistant Staphylococcus epidermidis was detected. *J Dermatol.* 2016; **43:** 460-2.

Hayashi M, Yanaba K, Umezawa Y, Asahina A, Nakagawa H. Impact of anti-tumor necrosis factor-alpha agents on serum levels of KL-6 and surfactant protein-D in patients with psoriasis. *J Dermatol.* 2017 Sep; **44:** 1063-6. Epub 2017 Mar 31.

Fukuda T, Umezawa Y, Tojo S, Yonenaga T, Asahina A, Nakagawa H, Fukuda K. Initial Experience of Using Dual-Energy CT with an Iodine Overlay Image for Hand Psoriatic Arthritis: Comparison Study with Contrast-enhanced MR Imaging. *Radiology.* 2017 Jul; **284:** 134-42. Epub 2017 Jan 3.

Nobeyama Y, Umezawa Y, Nakagawa H. Less-invasive analysis of DNA methylation using psoriatic scales. *J Dermatol Sci.* 2016; **83:** 70-3.

Salakhieva DV, Sadreev II, Chen MZ, Umezawa Y, Evstifeev AI, Welsh GI, Kotov NV. Kinetic regulation of multi-ligand binding proteins. *BMC Syst Biol.* 2016; **10:** 32.

Asahina A, Umezawa Y, Yanaba K, Nakagawa H. Serum C-reactive protein levels in Japanese patients with psoriasis and psoriatic arthritis: Long-term differential effects of biologics. *J Dermatol.* 2016; **43:** 779-84.

Yanaba K, Umezawa Y, Honda H, Sato R, Chiba M, Kikuchi S, Asahina A, Nakagawa H. Antinuclear antibody formation following administration of anti-tumor necrosis factor agents in Japanese patients with psoriasis. *J Dermatol.* 2016; **43:** 443-4.

Umezawa Y, Nakagawa H, Niiro H, Ootaki K; Japanese Brodalumab Study Group. Long-term clinical safety and efficacy of brodalumab in the treatment of Japanese patients with moderate-to-severe plaque psoriasis. *J Eur Acad Dermatol Venereol.* 2016; **30:** 1957-60.

Umezawa Y, Nakagawa H, Tamaki K. Phase III clinical study of maxacalcitol ointment in patients with palmoplantar pustulosis: A randomized, double-blind, placebo-controlled trial. *J Dermatol.* 2016; **43:** 288-93.

Yanaba K, Umezawa Y, Honda H, Sato R, Chiba M, Kikuchi S, Asahina A, Nakagawa H. Antinuclear antibody formation following administration of anti-tumor necrosis factor agents in Japanese patients with psoriasis. *J Dermatol.* 2016; **43:** 443-4.

Yanaba K, Asano Y, Akamata K, Noda S, Aozasa N, Taniguchi T, Takahashi T, Toyama T, Ichimura Y, Sumida H, Kuwano Y, Miyazaki M, Sato S. Circulating galectin-1 concentrations in systemic sclerosis: potential contribution to digital vasculopathy. *Int J Rheum Dis.* 2016; **19:** 622-7.

Maki T, Yanaba K, Ishiuji Y, Umezawa Y, Asahina A, Nakagawa H. Recurrent neutrophilic dermatosis of the face: a report of two ceses and review of the literature. *J Dermatol.* 2016; **43:** 811-4.

Yanaba K, Hayashi M, Yoshihara Y, Nakagawa H. Serum levels of soluble programmed death-1 and programmed death ligand-1 in systemic sclerosis: association with extent of skin sclerosis. *J Dermatol.* 2016; **43:** 954-7.

Shibata Y, Yanaba K, Ito K, Nishimura R, Miyawaki T, Nakagawa H. Nodular fasciitis on the face. *J Dermatol.* 2016; **43:** 1235-6.

Kubo N, Yanaba K, Kikuchi S, Fukuchi O, Nakagawa H, Namba H, Muro Y. Juvenile dermatomyositis positive for anti-DNA mismatch repair enzyme antibodies. *Eur J Dermatol.* 2017; **27:** 97-9.

Department of Radiology

Kunihiko Fukuda, *Professor*
Shunichi Sadaoka, *Professor*
Mayuki Uchiyama, *Professor*
Norio Nakata, *Associate Professor*
Masao Kobayashi, *Assistant Professor*
Toru Sakuma, *Assistant Professor*
Hiroshi Sekine, *Professor*
Manabu Aoki, *Professor*
Hiroya Ojiri, *Associate Professor*
Yoshimitsu Sunagawa, *Assistant Professor*
Mitsuko Ariizumi, *Assistant Professor*
Go Kawakami, *Assistant Professor*

Research Activities

Division of diagnostic imaging

1. Differentiating between glioblastomas with and without isocitrate dehydrogenase (IDH) gene mutant from the imaging findings

Along with the revision of the 2016 WHO classification of tumors of the central nervous system, molecular genetic parameters began to be used. In particular, the presence or absence of IDH gene mutation is one of the important elements in glioma classification. We examined whether the imaging findings can differentiate between glioblastomas with and without IDH gene mutation.

2. Evaluation of availability of Computed Tomography (CT) scoring system to detect metastatic nodal disease in patients with head and neck cancer

Nodal status is the most important prognosticator for patients with head and neck cancer. Although there are many CT criteria of metastatic adenopathy such as size, shape and internal morphology, how to determine positivity is quite subjective. We propose CT scoring system as an objective criterion and evaluate its availability.

3. Evaluation of the coronary vasculitis and peri-vasculitis using Electrocardiogram(ECG)-gated Multidetector row CT(MDCT)

The coronary vasculitis and perivasculitis are noticed as the complications of the various systemic disease: systemic vasculitis such as Takayasu arteritis、autoimmune disease such as systemic lupus erythematosus, lymphoproliferative disorder such as Immunoglobulin G(IgG)4-related disease, and so on. We examine the diagnostic utility of the ECG-gated MDCT in the evaluation of the coronary vasculitis and perivasculitis.

4. Airspace enlargement (AEF) is a recently recognized entity as a smoking related change, although the mechanism of forming thin-walled cysts are still unclear

We hypothesized that repeated transformation of cysts during respiration may one of the cause of forming AEF and compared the size change of cysts between AEF, bullae and honeycombing in respiration using inhalation and exhalation CT.

5. Evaluation of ultrasonic fusion images of breast lesions using Volume Navigation (V-NAVI)

It is possible to evaluate fusion images with ultrasonic / ultrasonic in real time during ultrasonic examination by using V-NAVI in which 3D-US images with GPS is acquired in advance. We evaluated both the fusion images of breast lesions and the utility of V-NAVI.

6. Evaluating the malignant potential of intraductal papillary mucinous neoplasms of the

pancreas (IPMN): Added value of non-enhanced endoscopic ultrasound to supplement non-enhanced MRI
Data from 38 patients histopathologically diagnosed with IPMN adenomas or IPMN adenocarcinomas were retrospectively analyzed. The diagnostic value of combined use of non-enhanced MRI and non-enhanced EUS were evaluated, as opposed to non-enhanced MRI alone.
7. MRI-US fusion imaging for the evaluation of placental invasion
We performed MRI-US fusion of the placenta in patients with suspected placental invasion, and explored the relation between MRI and US findings by MRI-US fusion.
8. Usefulness of Dual Energy CT (DECT) with Iodine MAP for the evaluation of hand psoriatic arthritis (PsA); comparison study with contrast enhanced MRI
To evaluate the usefulness of DECT for hand (PsA), we compared the DECT findings with contrast enhanced MRI.

Division of Ultrasound
9. Differentiation between benign and malignant breast lesions on contrast-enhanced sonography with quantitative analysis was performed
Kinetic analysis using contrast-enhanced sonography is useful for differentiation between benign and malignant breast lesions.

Division of Nuclear Medicine
10. Physiological change of accumulation in I-123 IMZ brain single-photon emission computed tomography (IMZ SPECT) appeared during childhood
Physiological regional accumulation shows change dramatically during childhood especially under 2 years old. The aim of this study was to compare regional accumulation in brain on anatomically standardized IMZ SPECT images.

Division of Interventional Radiology
11. Balloon-occluded Retrograde Transvenous Obliteration(BRTO) for gastric varices: Efficacy of Coaxial Double-balloon Catheter System (CDBCS)
CDBCS comprises a 9-Fr guiding balloon catheter that has a stiff shaft and a 5-Fr coaxial balloon catheter with a flexible shaft. In BRTO, this system can be more easily advanced proximally to the gastric varices beyond the outlet of the collateral veins. We investigated the efficacy of CDBCS compared with that of conventional single-balloon catheter system in BRTO of gastric varices.

Division of Radiation Therapy
12. Development of non-invasive quantitative evaluation for skin reaction of irradiation
Since the skin reactions associated with radiotherapy is quantitatively evaluated, objective evaluation is difficult between observers. The purpose of this study is to verify whether the change of skin reaction associated with radiotherapy using non-invasive quantitative methods objectively evaluate.

Publications

Yamauchi H, Buehler M, Goodsitt MM, Keshavarzi N, Srinivasan A. Dual-Energy CT-Based Differentiation of Benign Posttreatment Changes From Primary or Recurrent Malignancy of the Head and Neck: Comparison of Spectral Hounsfield Units at 40 and 70 keV and Iodine Concentration. *AJR Am J Roentgenol.* 2016; **206:** 580-7.

Baba A, Ojiri H, Dogru M, Tanaka Y, Takahashi S, Mogami T, Kobashi Y, Yamazoe S, Nozawa Y, Ogino N, Yamauchi H, Aoyagi Y. An Unusual Clinical Presentation of Kimura Disease Manifesting with a Typical Cephalocervical Lesion and an Atypical Subcutaneous Hip Mass Lesion. *Internal Medicine.* 2016; **55:** 1017-20.

Igarashi T, Ashida H, Morikawa K, Motohashi K, Fukuda K. Use of BI-RADS-MRI descriptors for differentiation between mucinous carcinoma and fibroadenoma. *Eur J Radiol.* 2016; **85:** 1092-8.

Ouchi K, Sakuma T, Kawai M, Fukuda K. Incidence and appearances of coronary sinus anomalies in adults on cardiac CT. *Jpn J Radiol.* 2016; **34:** 684-90.

Michimoto K, Shimizu K, Kameoka Y, Sadaoka S, Miki J, Kishimoto K. Transcatheter Arterial Embolization with a Mixture of Absolute Ethanol and Iodized Oil for Poorly Visualized Endophytic Renal Masses Prior to CT-Guided Percutaneous Cryoablation. *Cardiovasc Intervent Radiol.* 2016; **39:** 1589-94.

Fukuda T, Umezawa Y, Tojo S, Yonenaga T, Asahina A, Nakagawa H, Fukuda K. Initial experience of using Dual Energy CT with Iodine overlay image for hand psoriatic arthritis: comparison study with contrast enhanced MRI. *Radiology.* 2017; **284:** 134-42. Epub 2017 Jan 3.

Department of Surgery
Division of Digestive Surgery

Katsuhiko Yanaga, *Professor*
Norio Mitsumori, *Professor*
Hideyuki Kashiwagi, *Visiting Professor*
Masahiko Otsuka, *Visiting Professor*
Takeyuki Misawa, *Associate Professor*
Masaichi Ogawa, *Associate Professor*
Hidejirou Kawahara, *Associate Professor*
Sumio Takayama, *Associate Professor*
Minoru Matsuda, *Associate Professor*
Noburo Omura, *Associate Professor*
Yoshiyuki Hoya, *Associate Professor*
Naoto Takahashi, *Assistant Professor*
Shigeki Wakiyama, *Assistant Professor*
Ken Eto, *Assistant Professor*
Fumiaki Yano, *Assistant Professor*
Teruyuki Usuba, *Assistant Professor*
Atsuo Shida, *Assistant Professor*
Eiichiro Miura, *Assistant Professor*
Michiaki Watanabe, *Assistant Professor*
Kazuto Tsuboi, *Assistant Professor*
Kazuhiko Yoshida, *Professor*
Tomoyoshi Okamoto, *Professor*
Nobuyoshi Hanyu, *Visiting Professor*
Kenji Ikeuchi, *Visiting Professor*
Yoshio Ishibashi, *Associate Professor*
Yuichi Ishida, *Associate Professor*
Shuzo Kono, *Associate Professor*
Satoru Yanagisawa, *Associate Professor*
Yukio Nakabayashi, *Associate Professor*
Yoshiaki Tanabe, *Associate Professor*
Tomoyuki Tanaka, *Associate Professor*
Katsunori Nishikawa, *Assistant Professor*
Shuichi Fujioka, *Assistant Professor*
Yasuro Futagawa, *Assistant Professor*
Katsuhito Suwa, *Assistant Professor*
Hiroaki Shiba, *Assistant Professor*
Kaoru Mizusaki, *Assistant Professor*
Tetsuya Kajimoto, *Assistant Professor*
Tetsuya Kobayashi, *Assistant Professor*

General Summary

The delivery of research papers is supported by writing skills in addition to the ability to accomplish the study. More efforts to read scientific papers are necessary to improve writing skills and to ensure patient safety. All surgeons should keep in mind that research based on anatomic, pathologic, and physiologic principles, in combination with animal experimentation, makes it possible to develop complex operative procedures and to become the consummate surgeon, as stated in the last Southern Surgical Association Presidential Address (J Am Coll Surg 2015; 220(4); 387–395).

Research Activities

Upper gastrointestinal surgery

We evaluate the pathogenesis of primary esophageal motor functional disorders, especially achalasia and gastroesophageal reflux disease, using high-resolution manometry and multichannel intraluminal impedance pH monitoring. We have performed many laparoscopic operations and obtained good results. Recently, we introduced reduced port surgery and needlescopic surgery for minimally invasive surgery. Furthermore, we have started Per-Oral Endoscopic Myotomy (POEM) for achalasia since 2016. Taking into account the needs and pathophysiology, the best treatment for patients is selectively performed. Basic research in esophageal cancer led us to find molecular markers that indicate patients' prognoses. We aimed to investigate the significance of small ubiquitin-like modifier 1 (SUMO-1) expression in esophageal cancer as a prognostic factor. We found that

overexpression of SUMO-1 correlated with malignancy-associated pathological findings and poor prognoses. We continue to assess viability of the gastric tube using intraoperative thermal imaging system during esophagectomy. Correlation between suitable graft construction and postoperative complications of a graft was then investigated. We also continue to examine intraoperative recurrent nerve monitoring in order to prevent postoperative recurrent nerve palsies as well as to predict degree of paralysis after surgery. We started to analyze upper esophageal sphincter and residual esophageal motility since 2016 by high resolution manometry. We started providing minimum invasive surgery for patients with early gastric cancer through sentinel node navigation surgery (SNNS) in 2001. We have conducted more than 300 cases of such operations until now. We developed SNNS using infrared ray endoscopic system for the first time in the world. In addition, we have conducted surveying biological cancer behavior using immunohistochemical and RT-PCR methods, which revealed that zinc finger protein (ZNF) 217 was an independent prognostic factor for relapse-free survival and a novel prognostic biomarker for patients with gastric cancer. Postgastrectomy syndrome comprises specific symptoms after gastrectomy and is a target for treatment. To decrease the incidence and severity of postgastrectomy syndrome and to maximize residual gastric function, several types of limited gastric resection with refined techniques of reconstruction have been attempted. In addition, after gastrectomy, multiple tests of postoperative gastrointestinal function are performed to evaluate various types gastrectomy procedures. We also started sleeve gastrectomy for morbid obesity.

Lower gastrointestinal surgery

We have reported the beneficial cosmetic outcomes of a novel and patient-friendly ileostomy procedure. This procedure uses the umbilical fossa for placement of a defunctioning ileostomy followed by a simple umbilicoplasty for ileostomy closure. In collaboration with the Department of Internal Medicine we hold conference and register the database of chemotherapy to examine combined therapy for the colorectal cancer. We started studies on anal function using Stationary 3D-manometory. Together with the Department of Biochemistry we are committed to construct a complementary DNA library from the surgical specimens of colorectal cancer to analyze the expression of intracellular signal molecules that are associated with progression and growth of cancer. As a first step of the project, the following basic research will be started: analysis of the cell-cycle regulation and dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2(DYRK2) in relation to c-jun/c-myc phosphorylation. By correlating with the clinical database the relationship between the stage of colorectal cancer and the manifestation of DYRK2 and associated genes is investigated. When chemoradiation therapy is performed for rectal cancer, radiation causes microenvironmental inflammation around cancer cells and promotes the secretion of matrix metalloproteinase (MMP) and nuclear factor kapp B (NF-κB). In addition, NF-κB is reported to directly induce MMP. The basement membrane is dissolved by MMP, and cancer cells enter the bloodstream and metastasize to other organs. Therefore, suppression of MMP might prevent metastasis after surgery. We will examine whether down regulation of NF-kB decreases the recurrence and metastasis of colon cancer.

Hepatobiliary and pancreatic surgery

The first LDLT was successfully performed for a patient with postnecrotic cirrhosis and HCC in February 2007. Our first blood type ABO-incompatible LDLT (15th LDLT) was performed for a patient with primary biliary cirrhosis in June 2015. Our 20th LDLT was performed for a patient with primary biliary cirrhosis in March 2017. All 20 recipients were discharged in good condition on postoperative day 15 to 70, and donors were discharged on postoperative day 7 to 26 and returned to preoperative status. We are planning to extend the indication of LDLT to acute hepatic failure. The 5-year cumulative overall survival rate of HCC after hepatic resection in our department is 75%, which is significantly better than the mean survival rate in Japan (56.8%). We have performed clinical trials for pancreatic cancer and biliary tract cancer. Ongoing trials for pancreatic cancer are evaluating combination chemotherapy with gemcitabine, S-1 with regional arterial infusion of nafamostat mesilate for advanced pancreatic cancer, and gemcitabine in combination with regional arterial infusion of nafamostat mesilate as an adjuvant chemotherapy. A current trial for advanced biliary tract cancer is evaluating chemotherapy with S-1 every other day in combination with gemcitabine/cisplatin. We have also performed extended liver resections as a conversion therapy for multiple metastatic tumors of the liver, mainly originating from colorectal cancers. Furthermore, laparoscopic surgery, including hand-assisted laparoscopic surgery and laparoscopy-assisted, i.e., hybrid surgery, has gradually been expanded for hepatobiliary, pancreatic, and splenic diseases because of its lower invasiveness. Navigation for liver resection has been paid for by national health insurance since April 1, 2012, and the Vincent navigation system was introduced in July 2012. Biliary and pancreatic navigation surgery for either open or laparoscopic surgery is performed with the Institute for High Dimensional Medical Imaging Research Center. With regard to nutritional therapy for patients who have cancer, clinical and experimental studies are examining enhanced recovery after surgery, surgical site infection, and the use of eltrombopag before laparoscopic splenectomy for idiopathic thrombocytonpenic purpura.

Publications

Hoya Y, Taki T, Watanabe A, Nakayoshi T, Okamoto T, Mitsumori N, Yanaga K. Durable flap-valve mitigation of duodeno-gastric reflux, remnant gastritis and dumping syndrome following Billroth I reconstruction. *J Gastrointest Surg.* 2016; **20:** 772-5.

Nishikawa K, Fujita T, Yuda M, Yamamoto SR, Tanaka Y, Matsumoto A, Tanishima Y, Yano F, Mitsumori N, Yanaga K. Early postoperative endoscopy for targeted management of patients at risks of anastomotic complications after esophagectomy. *Surgery.* 2016; **160:** 1294-301.

Takahashi N, Nimura H, Fujita T, Mitsumori N, Shiraishi N, Kitano S, Satodate H, Yanaga K. Laparoscopic sentinel node navigation surgery for early gastric cancer: a prospective multicenter trial. *Langenbecks Arch Surg.* 2017; **402:** 27-32.

Yano F, Omura N, Tsuboi K, Hoshino M, Yamamoto SR, Akimoto S, Masuda T, Mitsumori N, Kashiwagi H, Yanaga K. Outcomes of redo surgery for failed laparoscopic fundoplication. *Esophagus.* 2016; **13:** 290-4.

Tsuboi K, Omura N, Yano F, Hoshino M, Yamamoto SR, Akimoto S, Masuda T, Kashiwagi H, Yanaga K. Identification of risk factors for mucosal injury during laparoscopic myotomy for achalasia. *Surg Endosc.* 2016; **30:** 706-14.

Tsuboi K, Omura N, Yano F, Hoshino M, Yamamoto SR, Akimoto S, Masuda T, Kashiwagi H, Yanaga K. Gender differences in both the pathology and surgical outcome of esophageal achalasia patients. *Surg Endosc.* 2016; **30:** 5465-71.

Shida A, Mitsumori N, Fujioka S, Takano Y, Iwasaki T, Takahashi N, Ishibashi Y, Omura N, Yanaga K. Comparison of short-term and long-

term clinical outcomes between laparoscopic and open total gastrectomy for patients with gastric cancer. *Surg Laparosc Endosc Percutan Tech.* 2016; **26:** 319-23.

Aoki H, Aoki M, Katsuta E, Ramanathan R, Idowu MO, Spiegel S, Takabe K. Host sphingosine kinase 1 worsens pancreatic cancer peritoneal carcinomatosis. *J Surg Res.* 2016; **205:** 510-7.

Matsumoto A, Watanabe M, Mine S, Nishida K, Shigaki H, Kawabata K, Yanaga K, Sano T. Comparison of synchronous versus staged surgeries for patients with synchronous double cancers of the esophagus and head-and-neck. *Dis Esophagus.* 2017; **30:** 1-6.

Hoshino M, Omura N, Yano F, Tsuboi K, Yamamoto SR, Akimoto S, Kashiwagi H, Yanaga K. Backflow prevention mechanism of laparoscopic Toupet fundoplication using high-resolution manometry. *Surg Endosc.* 2016; **30:** 2703-10.

Konishi H, Nakada K, Kawamura M, Iwasaki T, Murakami K, Mitsumori N, Yanaga K. Impaired gastrointestinal function affects symptoms and alimentary status in patients after gastrectomy. *World J Surg.* 2016; **40:** 2713-8.

Taki T, Hoya Y, Watanabe A, Nakayoshi T, Okamoto T, Sekine H, Mitsumori N, Yanaga K. Usefulness of chemoradiotherapy for inoperable gastric cancer. *Ann R Coll Surg Engl.* Epub 2016 Sep.

Fujisaki M, Shinohara T, Hanyu N, Kawano S, Tanaka Y, Watanabe A, Yanaga K. Laparoscopic gastrectomy for gastric cancer in the elderly patients. *Surg Endosc.* 2016; **30:** 1380-7.

Akimoto S, Singhal S, Masuda T, Yamamoto SR, Svetanoff WJ, Mittal SK. Esophagogastric Junction Morphology and Distal Esophageal Acid Exposure. *Dig Dis Sci.* 2016; **61:** 3537-44.

Kawahara H, Watanabe K, Hojo S, Ishida K, Misawa T, Akiba T, Yanaga K. Feasibility of single-incision laparoscopic surgery plus one assist port for anterior resection. *Anticancer Res.* 2016; **36:** 467-70.

Ogawa M, Watanabe M, Hasegawa T, Ichihara K, Yoshida K, Yanaga K. Expression of CXCR-4 and IDO in human colorectal cancer: An immunohistochemical approach. *Mol Clin Oncol.* 2017; **6:** 701-4.

Suwa K, Nakajima S, Uno Y, Suzuki T, Sasaki S, Ushigome T, Eto K, Okamoto T, Yanaga K. Laparoscopic modified Sugarbaker parastomal hernia repair with 2-point anchoring and zigzag tacking of Parietex™ Parastomal Mesh technique. *Surg Endosc.* 2016; **30:** 5628-34.

Eto K, Kosuge M, Ohkuma M, Haruki K, Neki K, Mitsumori N, Ishida K, Yanaga K. Comparison of transumbilical and conventional defunctioning ileostomy in laparoscopic anterior resections for rectal cancer. *Anticancer Res.* 2016; **36:** 4139-44.

Fujioka S, Misawa T, Yanaga K. Isolating tape method is useful for an early judgment of curability during pancreaticoduodenectomy for pancreatic cancer. *J Hepatobiliary Pancreat Sci.* 2016; **23:** E20-4.

Usuba T, Misawa T, Ito R, Yoshida K, Hanyu N, Yanaga K. Safety of no-stented pancreaticojejunostomy in pancreaticoduodenectomy for patients with soft pancreas. *Anticancer Res.* 2016; **36:** 6619-23.

Shiba H, Hashimoto K, Kelly DM, Fujiki M, Quintini C, Aucejo F, Diago T, Yerian L, Yanaga K, Matsushima M, Eghtesad B, Fung JJ, Miller C. Risk stratification of allograft failure secondary to hepatitis C recurrence after liver transplantation. *Hepatol Res.* 2016; **46:** 1099-106.

Sakamoto T, Ishii Y, Shiba H, Furukawa K, Fujiwara Y, Haruki K, Iwase Y, Shirai Y, Yanaga K. Inhibitory effect of anti-rheumatic drug iguratimod for hepatocellular carcinogenesis by inhibition of serum interleukin-8 production. *Anticancer Res.* 2016; **36:** 3301-6.

Furukawa K, Gocho T, Shirai Y, Iwase R, Haruki K, Fujiwara Y, Shiba H, Misawa T, Yanaga K. The Decline of Amylase Level of Pancreatic Juice After Pancreaticoduodenectomy Predicts Postoperative Pancreatic Fistula. *Pancreas.* 2016; **45:** 1474-7.

Haruki K, Shiba H, Shirai Y, Horiuchi T, Iwase R, Fujiwara Y, Furukawa K, Misawa T, Yanaga K. The C-reactive protein to albumin ratio predicts long-term outcomes in patients with pancreatic cancer after pancreatic resection. *World J Surg.* 2016; **40:** 2254-60.

Haruki K, Shiba H, Horiuchi T, Sakamoto T, Gocho T, Fujiwara Y, Furukawa K, Misawa T, Yanaga K. Impact of the C-reactive protein to albumin ratio on long-term outcomes after hepatic resection for colorectal liver metastases. *Am J Surg*. 2017 Feb 6. [Epub ahead of print]

Shirai Y, Shiba H, Iwase R, Haruki K, Fujiwara Y, Furukawa K, Uwagawa T, Ohashi T, Yanaga K. Dual inhibition of nuclear factor kappa-B and Mdm2 enhance the antitumor effect of radiation therapy for pancreatic cancer. *Cancer Lett.* 2016; **370:** 177-84.

Shirai Y, Uwagawa T, Shiba H, Shimada Y, Horiuchi T, Saito N, Furukawa K, Ohashi T, Yanaga K. Recombinant thrombomodulin suppresses tumor growth of pancreatic cancer by blocking thrombin-induced PAR1 and NF-κB activation. *Surgery.* 2017 Jan 13. [Epub ahead of print]

Horiuchi T, Uwagawa T, Shirai Y, Saito N, Iwase R, Haruki K, Shiba H, Ohashi T, Yanaga K. New treatment strategy with nuclear factor-κB inhibitor for pancreatic cancer. *J Surg Res.* 2016; **206:** 1-8.

Reviews and Books

Shida A, Mitsumori N, Nimura N, Takano Y, Iwasaki T, Fujisaki M, Takahashi N, Yanaga K. Prediction of lymph node metastasis and sentinel

node navigation surgery for patients with early-stage gastric cancer. *World J Gastroenterol.* 2016; **22:** 7431-9.

Suwa K, Okamoto T, Yanaga K. Closure versus non-closure of fascial defects in laparoscopic ventral and incisional hernia repairs: a review of the literature. *Surg Today.* 2016; **46:** 764-73.

Department of Surgery
Division of Chest Surgery, Breast and Endocrinology Surgery

Chest Surgery
Toshiaki Morikawa, *Professor*
Tadashi Akiba, *Professor*
Shuji Sato, *Associate Professor*
Makoto Odaka, *Associate Professor*

Breast and Endocrine Surgery
Hiroshi Takeyama, *Professor*
Satoki Kinoshita, *Professor*
Yasuo Toriumi, *Professor*
Kazumi Kawase, *Associate Professor*
Isao Tabei, *Associate Professor*
Hiroko Nogi, *Assistant Professor*

General Summary

The Divisions of Chest Surgery and of Breast and Endocrinology Surgery were established in June 2005. Since then, all staff members have been active in surgical practice, research, and education. Many studies are ongoing.

Research Activities

Chest Surgery

Thoracoscopic surgery is the focus of our clinical activity. This minimally invasive surgery produces fewer postoperative complications and sequelae and is especially beneficial for elderly, high-risk patients. Thoracoscopic surgery requires advanced skills, and we have independently developed total thoracoscopic surgery, which uses only a thoracoscope and video monitors to provide intraoperative views. Our method of thoracoscopic surgery can be used to treat many chest conditions, such as juvenile pneumothorax, peripheral lung nodules, mediastinal tumors, and lung cancer.

Thoracoscopic surgery is also indicated for higher-risk patients with such complications as advanced pulmonary emphysema, impaired pulmonary function, and extremely high age who are not candidates for conventional open surgery.

Operative procedures, including wedge resection, segmentectomy, lobectomy, and pneumonectomy of the lung, are all safely performed, in addition to resection of mediastinal tumors or the thymus. Surgery for lung cancer requires much more advanced skills and oncological considerations, which have also been independently developed. Of the mediastinal procedures, thymectomy is usually performed via thoracoscopy rather than via a conventional median sternotomy. In our department the percentage of the chest operations performed via thoracoscopy is more than 90%, which we assume to be the highest rate in the world.

The minimal invasiveness of thoracoscopic surgery is being investigated with prospective clinical studies. These studies include a comparative study of open surgery and video-assisted surgery for lung cancer and evaluations of video-assisted surgery for bullous lung diseases in elderly patients with impaired lung function, of video-assisted surgery for thymic tumors, and of video-assisted thymectomy for myasthenia gravis.

Our clinical studies are also evaluating new devices and methods, such as narrow-band imaging for the thoracoscopic diagnosis of benign and malignant lung diseases, and LaparoSonic coagulating shears (Ethicon Endo-Surgery, Inc., Cincinnati, OH, USA) for small thoracotomy. Three-dimensional diagnosis with computed tomography is used to make thoracoscopic surgery safer. The diagnosis and treatment of ground glass opacity of the lung, which is considered to indicate early adenocarcinoma, are being evaluated.
Many basic research studies are also underway. In the morphological expression-related advancement of the molecular genetic analysis of lung cancer, we are investigating whether carcinogenesis of the lung as reflected by CA19-9 activity is an important marker of de novo carcinogenesis. The biological and genetic characteristics of peripheral adenocarcinoma of the lung are being investigated to establish the most appropriate surgical procedures. Correlation with the detection of blood CTC (Circulating Tumor Cells) and the prognosis of the patient with lung cancer is examined.
The oncogene of the lung cancer is analyzed with the next generation sequencer.
A system for viewing videos on the Internet is now being developed which will help improve surgical training and research.

Breast
1. Clinical study
1) The evaluation of sentinel node biopsy after neoadjuvant chemotherapy
The minimally invasive technique of sentinel lymph node biopsy produces less morbidity and allows accurate pathologic staging of the axilla. Experience with sentinel node biopsy after neoadjuvant chemotherapy is limited. The purpose of our clinical study is to evaluate the accuracy and safety of this procedure in breast cancer patients after neoadjuvant chemotherapy.
2) Evaluation of the effectiveness of exercise for psychiatric illness in patients after surgery for breast cancer
Many woman experience the psychiatric illness such as emotional distress, depression and anxiety after a diagnosis of breast cancer. We have prospectively investigated the effects of exercise on psychological health.
3) Cryotherapy for small breast cancer
Cryoablation using extremely cold temperatures to destroy tumor tissues has been increasingly recognized as a highly efficient cancer therapy. Therefore, cryotherapy has been used in the clinic to treat several types of tumors, including breast, kidney, liver, esophagus, skin, prostate, lung and bone. Moreover, the efficiency of cryotherapy, combined with its limited side-effects, has been confirmed through pilot studies with early-stage breast cancer patients.
4) Therapeutic strategy for oligometastatic breast cancer
We have analyzed the patients with metastatic breast cancer for thirty years. The analyses indicate that oligometastatic breast cancer is a distinct subgroup with long-term prognosis superior to metastatic breast cancer. We try further prospective studies to better characterize oligometastatic breast cancer to improve prognosis in metastatic breast cancer.
2. Basic research
1) Development of breast cancer

We have investigated biological factors involved in the progression of carcinoma in situ to invasive breast cancer using the immunohistochmeical techniques.
2) Clinically useful biomarkers for triple negative breast cancer
Triple negative breast cancer is heterogeneous disease. We have investigated prognostic and predictive biomarkers for triple negative breast cancer.
3) CTC and DTC
We had investigated clinical values of the presence of circulating tumor cells in the peripheral blood and disseminated tumor cells in the bone marrow (DTC-BM) of patients with early breast cancer. Detection of DTC-BMs was useful for observing adjuvant therapy effects and for predicting relatively late-phase metastasis. The cluster status of CTCs suggested early relapsing.
4) Cancer stem-cell
We evaluated the association of presence of cancer stem cell between primary breast tumor and metastatic site and the correlation with prognosis.
5) Dual Specificity Tyrosine Phosphorylation Regulated Kinase 2 (DYRK2)
We have evaluated the association between DYRK2 and stem cell or mTOR signal. The ectopic expression of DYRK2 promoted phosphorylation of Thr631 for the ubiquitination and degradation of mTOR. DYRK2 expression levels might thus predict clinical responses to everolimus. Furthermore, DYRK2 was a novel negative regulator for formation of breast cancer stem cells. Downregulation of DYRK2 promoted cancer stem-like traits in vitro, tumourigenesis in vivo and the proportion of the cancer stem cell population in human breast cancer tissues. We found that Krupple-like factor 4 (KLF4) serves as a key mediator of DYRK2's control over the cancer stem phenotype. Reduced DYRK2 expression increased KLF4 expression, which induced cancer stem-like properties. We identified androgen receptor (AR) as a transcription factor binding to the KLF4 promoter region; this process was dependent on DYRK2 kinase activity. Our findings delineated a mechanism of cancer stem cell regulation by the DYRK2-AR-KLF4 axis in breast cancer. Targeting of this pathway might be a promising strategy against breast cancer stem cells.

Endocrine
Clinical research
Lenvatinib is one of tyrosine kinase inhibitor, which blocks VEGF Receptor1-3kinase. Lenvatinib had developed in Japan, and prolonged the progression free survival (PFF) to, in phase III trial.
We are planning and going a multi-center clinical trial for thyroid carcinomas, including papillary carcinoma, medullary carcinoma, and undifferentiated carcinoma to confirm the antitumor effects of Lenvatinib in thyroid carcinoma treated major facilities of Japan from 2016.
Our department is joining to this trial from 2016.

Basic research
The detection of antigens of thyroid carcinoma in sera.
A monoclonal antibody, designated JT-95, was made against a thyroid papillary carci-

noma obtained by our Department of Breast and Endocrine Surgery. We are attempting to measure the antigen recognized by JT-95 in the serum of patients with papillary carcinoma, in collaboration with the Molecular Cell Biology Division of Jikei University. The quantity of antigen of JT-95 is higher in patients with papillary carcinoma, especially those with metastasis to lung or bone, than in patients with breast carcinoma.
We are now trying to make chromatography of JT-95 to detect thyroid carcinoma antigen more easily.

Publications

Odaka M, Shibasaki T, Kato D, Mori S, Asano H, Yamashita M, Morikawa T. Comparison of oncological results for early- and advanced-stage thymomas: thoracoscopic thymectomy versus open thymectomy. *Surg Endosc.* 2017; **31:** 734-42.

Odaka M, Tsukamoto Y, Shibasaki T, Katou D, Mori S, Asano H, Yamashita M, Morikawa T. Thoracoscoic thymectomy is a feasible and less invasive alternative for the surgical treatment of large thymomas. *Interact Cardiovasc Thorac Surg.* 2017; **25:** 103-8. Epub 2017 Mar 23.

Mori S, Motoi N[1], Ninomiya H[1], Matsuura Y[1], Nakao M[1], Mun M[1], Okumura S[1], Nishio M[1], Morikawa T, Ishikawa Y[1] ([1]The Cancer Institute Hospital). High expression of programmed cell death 1 ligand 1 in lung adenocarcinoma is a poor prognostic factor particularly in smokers and wild-type epidermal growth-factor receptor cases. *Pathol Int.* 2017; **67:** 37-44.

Takeyama H, Shimada T, Kinoshita S, Uchida K. Usefulness of CTC and DTC-BM Detection for Adjuvant Therapy Effects and Prognosis Prediction in Early Breast Carcinoma: Results of 8-11 Years of Follow-up Evaluation. *Ann Surg Oncol.* 2017; **24:** 1227-33. Epub 2016 Dec 1.

Kinoshita S, Fukushima N, Miyake R, Ishigaki T, Hirano A, Mimoto R, Uchida K, Takeyama H, Morikawa T. Clinicopathological assessment of patients with locally advanced breast cancer with 10 or more lymph node metastases. *Breast Can Curr Res.* 2016; **1:** 107.

Kinoshita S, Nojima K, Miyake R, Shimada N, Hirano A, Uchida K, Takeyama H, Morikawa T. Clinical assessment of skin incisions used for skin-sparing mastectomy. *Intern Med Rev.* 2016; **2:** 197.

Kawase K, Carpelan-Holmström M (Helsinki University Central Hospital), Kwong A (The University of Hong Kong), Sanfey H (SIU School of Medicine). Factors that can promote or impede the advancement of women as leaders in surgery: results from an international survey. *World J Surg.* 2016; **40:** 258-6.

Yorozuya K[1], Kawase K, Akashi-Tanaka S[2], Kanbayashi C[3], Nomura S[4], Tomizawa Y[5] ([1]Kawasaki Municipal Hospital, [2]Showa University Hospital, [3]Niigata Cancer Center Hospital, [4]Univ Tokyo, [5]Tokyo Women's Medical Univ). Mentorship as Experienced by Women Surgeons in Japan. *World J Surg.* 2016; **40:** 38-44.

Reviews and Books

Odaka M, Shibasaki T, Kato D, Mori S, Asano H, Yamashita M, Morikawa T. Surgical and oncological outcomes of thoracoscopic thymectomy for thymoma. *J Vis Surg.* 2017; **3:** 54.

Kinoshita S, Nojima K, takeyama H, Uchida K. Four types of skin incisions for skin-sparing mastectomy with immediate breast reconstruction. In: Shiffman MA, editor. Breast Reconstruction: art, science, and new clinical techniques. New York: Springer; 2016. p. 547-54.

Department of Surgery
Division of Pediatric Surgery and Vascular Surgery

Takao Ohki, *Professor and Chairperson*
Yuji Kanaoka, *Associate Professor*
Joji Yoshizawa, *Assistant Professor*
Atsushi Ishida, *Associate Professor*
Naoki Toya, *Assistant Professor*

General Summary

Pediatric Surgery
Surgery for children at The Jikei University Hospital is offered by a highly trained, expert team of pediatric surgical professionals who specialize in the diseases and conditions affecting young people. Our surgeons work exclusively with infants, children and adolescents and understand their unique needs.

Vascular Surgery
Research projects of our department have focused on the development of the endovascular repair of aortic aneurysms, treatment of peripheral arterial disease with new techniques and devices including clinical trials.

Research Activities

Pediatric Surgery
1. Education
Education for medical students: The patients with pediatric surgery have congenital anomaly. The lecture of pediatric surgery for students is based on embryology.
Education for training doctors: Three objects for training doctor in pediatric surgery are: 1) How to obtain a blood sample from pediatric patients, 2) Understanding about fluid therapy for pediatric patients, 3) Learning the way of buried suture.
Education for surgical residents: They are able to operate as operator or assistant for pediatric surgery.
2. Clinical study
Minimally invasive and scarless surgeries. That's how we make our mark.
1) Endoscopic treatment for vesicoureteral reflux using Deflux®
There are three options for managing or treating vesicoureteral reflux. We select the endoscopic treatment with Deflux. We have treated three cases, two cases were treated completely.
2) Laparoscopic percutaneous extraperitoneal closure for inguinal hernia: learning curve for attending surgeons and residents
Laparoscopic percutaneous extraperitoneal closure (LPEC) for pediatric inguinal hernia is a simple technique in which a purse‐string suture made of nonabsorbable material is placed extraperitoneally around the hernia orifice by a special suture needle (Lapaherclosure™). Concerns have been raised about the extensive learning curve for both attending

surgeons and residents to master this technique. This study assesses the difference in learning curves for the safe performance of LPEC by attending surgeons and residents.
3) The Nuss procedure also aims to force the sternum forward and hold it there with an implanted steel bar, but without making a big incision to resect the abnormal cartilage. In this procedure, the curved steel bar is placed under the sternum through two small incisions on the sides of the chest. No. 3 in Japan; The number of surgical patients with pectus excabatum is the best three in Japan.
3. Basic study
1) MicroRNAs transported by exosomes in body fluids as mediators of intercellular communication in human neuroblastoma. Cancer-cell communication is an important and complex process, achieved through a diversity of mechanisms that allows tumor cells to mold and influence their environment. In recent years, evidence has accumulated indicating that cells communicate via the release and delivery of microRNAs (miRNAs) packed into tumor-released (TR) exosomes. Understanding the role and mode of action of miRNAs from TR exosomes is of paramount importance in the field of cancer biomarker discovery and for the development of new biomedical applications for cancer therapeutics.

Vascular Surgery
1. Development of endovascular repair of thoracoabdominal aneurysms
Although stent grafts for the treatment of abdominal aortic aneurysms (AAAs) have been developed and are commercially available, no such stent grafts are available for the treatment of thoracoabdominal aortic aneurysms (TAAAs) in Japan. The surgical death rate following open surgery for the treatment of AAAs is satisfactory, but that for the treatment of TAAAs remains unacceptably high at 15% to 20%, and further improvement is desperately needed. Because a TAAA involves 1 or more visceral arteries, visceral perfusion must be maintained while the aneurysm is excluded with stent grafts. We have used a custom-made t-Branch stent graft in combination with covered stents (for visceral reconstruction) for the treatment of TAAAs that were considered inoperable because of comorbid conditions or a hostile thorax/abdomen after an approval of IRB. Although stent graft repair for TAAAs requires long operative and fluoroscopic time, this treatment is feasible and safe.
2. Development of endovascular repair of aortic arch aneurysms: Retrograde in-situ branched surgery; Branched Thoracic Arch stent grafts
We have developed a new minimally invasive operation for aortic arch aneurysms. After carotid-carotid bypass surgery if needed is performed and stent grafts are placed, a needle is used to prick the stent graft thorough one side of a carotid artery, after which a covered stent is inserted as a branch and deployed into the stent graft (in an in-situ retrograde fashion). We have examined this retrograde in-situ branched surgery in an in-vitro study and have applied it clinically. This operation is expected to be a less invasive surgery for aortic arch aneurysms. We also use Branched Thoracic Arch stent grafts those are commercially available in Europe for endovascular repair of aortic arch aneurysms after an approval of IRB.
3. Research on drug-eluting stent in the superficial femoral artery
The Zilver PTX drug-eluting peripheral stent (Cook Medical, Bloomington, IN, USA) is

specifically designed and approved to treat peripheral arterial disease affecting the superficial femoral artery, the main vessel of the thigh. The Zilver PTX is a self-expanding stent made of nitinol, a space-age "shape memory" metal that offers unique mechanical advantages for a stent in the superficial femoral artery.

Both a global registry and a randomized controlled trial, in which most patients were enrolled in the United States, but also in Germany and Japan. We participated in this trial. After reviewing its 1-year primary endpoint, the Zilver PTX received approval from the Japanese Pharmaceuticals and Medical Devices Agency in January 2012 and is now commercially available in Japan.

Publications

Ohki T. Zilver PTX Post-Market Surveillance Study of Paclitaxel-Eluting Stents for Treating Femoropopliteal Artery Disease in Japan: 12-Month Results. *JACC Cardiovasc Interv.* 2016; **9:** 271-7.

Kanaoka Y, Ohki T, Ozawa H. Repair of chronic aneursymal aortic dissection using a stent graft and an Amplatzer vascular plug: a case study. *Ann Vasc Surg.* 2017 Feb; **39:** 288.e5-12. Epub 2016 Sep 23.

Kanaoka Y, Ohki T, Maeda K, Baba T. Analysis of risk factors for early type 1 endoleaks after thoracic endovascular aneurysm repair. *J Endovasc Ther.* 2017 Feb; **24:** 89-96. Epub 2016 Oct 22.

Kanaoka Y, Ohki T, Maeda K, Baba T, Fujita T. Multivariate analysis of risk factors of cerebral infarction in 439 patients undergoing thoracic endovascular aneurysm repair. *Medicine (boltimore).* 2016 Apr; **95:** e3335.

Toya N, Ohki T, Momokawa Y, Shukuzawa K, Fukushima S, Tachihara H, Akiba T. Risk factors for early renal dysfunction following endovascular aortic aneurysm repair and its effect on the postoperative outcome. *Surg Today.* 2016 Dec; **46:** 1362-9.

Kanamori D, Yoshizawa J. A review of laparoscopic percutaneous extra peritoneal closure (LPEC) for the treatment of pediatric inguinal hernia. *J Surg Transplant Sci.* 2016; **4:** 1029-32.

Kurobe M, Baba Y, Otsuka M. Inguinal hernia in very low-birthweight infants: Follow up to adolescence. *Pediatr Int.* 2016; **58:** 1322-7.

Department of Orthopaedic Surgery

Keishi Marumo, *Professor*
Hajime Sugiyama, *Professor*
Shigeru Soshi, *Associate Professor*
Makoto Kubota, *Associate Professor*
Mitsuru Saito, *Associate Professor*
Soki Kato, *Assistant Professor*
Tetsuro Nishizawa, *Assistant Professor*
Takuya Otani, *Professor*
Takaaki Tanaka, *Professor*
Hiroki Funasaki, *Associate Professor*
Mamoru Yoshida, *Associate Professor*
Hideki Fujii, *Assistant Professor*
Ryo Ikeda, *Assistant Professor*

General Summary

Basic Research
Our studies on bone metabolism and osteogenesis have been highly acclaimed both in Japan and abroad. Our research on mobility at the intercuneiform joint has played a pioneering role in the field of foot surgery. Our clinical research focuses on the relationship between systemic disorders, such as life-style related diseases and aging, and bone disease.

Clinical Research
Our clinical practice has been divided into 10 subspecialties to treat a wide range of musculoskeletal disorders and is managed by different specialist teams: knee joint, hip joint, spine, shoulder joint, foot surgery, trauma, osteoporosis, rheumatic diseases and sports. All teams maintain a high level of expertise and are actively involved in scientific activities.

Research Activities

Long-term results of Modified Inferior Capsular Shift procedure (MICS) for recurrent dislocation of the shoulder
Long-term clinical results of modified inferior capsular shift (MICS) procedure for recurrent dislocation of the shoulder were reviewed. The study consisted of 17 patients with their average age at surgery of 30 years (follow-up ratio, 47%), and an average follow-up period of 12 years and 6 months. The recurrence was observed in one judo teenage player. The ratio of returning to previous sports activity was 94%. No recurrence was observed in patients who had a large Hill-Sachs lesion or general joint laxity. MICS procedure including capsular shift with 30 to 40 degrees of external rotation produced satisfactory long-term results with less limitation in range of motion.

Current developments and ongoing studies
Apart from Dupuytren's contractures, we treat many other diseases: from traumatic injuries such as fractures, tendon ruptures and neurovascular injuries to degenerative disorders and tumors. Additionally, we perform highly specialist surgical procedures including tendon suturing and microsurgery.

Sacroiliac joint (SIJ) degeneration in elderly patients who underwent surgical treatment for a lumbar lesion

We evaluated a degree of sacroiliac joint (SIJ) degeneration using CT images in elderly patients who had undergone surgical treatment for a lumbar lesion. In over 90% of patients, some degree of SIJ degeneration was observed. It was particularly strong in patients with hyperostotic changes in the thoracic vertebrae where SIJ degenerations were noticeable, and in some cases it included SIJ ankylosis.Our findings suggest that hereditary and/or biomechanical factors affect degeneration of the sacroiliac joint.

Two-stage treatment of chronic peri-prosthetic joint infection with retention of a well-fixed and well-functioning cement-less stem

The clinical course of 6 patients treated for chronic peri-prosthetic joint infections (PJI) without stem removal was examined. The first-stage surgery involved acetabular cup removal and reconstruction by filling the acetabular defect with antibiotic-loaded acrylic cement and creating a socket-like hemispherical dent. After confirming that infection had been eradicated, the second-stage acetabular reconstruction was performed. Results: Patients underwent active range-of-motion and ambulation exercises between two surgeries. One patient died of an unrelated non-infective cause 1 year postoperatively; 5 patients had good functional outcomes and radiographic findings with no PJI recurrence.

Accuracy of CT-Based Navigation-Assisted Total Knee Arthroplasty and Anterior Cruciate Ligament reconstruction with an originally designed retro-rectangle dilator

CT-based navigation system (CTNS) is one of the computer-assisted surgical systems available for total knee arthroplasty. We focused on the accuracy of component orientation and postoperative alignment with CTNS compared to a conventional technique using outliers data. Our results demonstrated that CTNS significantly improved both parameters.

We developed a new technique of making bone tunnels with a rectangular retro-dilator. Although only few patients have been followed-up for sufficient periods, our procedure provides safer and easier approach for anatomical rectangular anterior cruciate ligament reconstructions.

Evaluation of mobility at the articulation between medial and middle cuneiforms using a 3D analysis system and weightbearing CT in normal versus hallux valgus patients

We measured the three-dimensional displacement of the middle cuneiform relative to the medial cuneiform under weightbearing conditions and compared data between hallux valgus and control groups. Displacement by dorsiflexion and inversion was significantly greater in the hallux valgus group. It may be possible to further improve postoperative outcomes of the Lapidus procedure through arthrodesis of the intercuneiform 1-2 joint also in patients with severe hypermobility of this joint.

Bone metabolism team: current developments and ongoing studies

In our outpatient clinic specializing in bone metabolism, we provide personalized treatments using simultaneous estimation of bone quantity and bone quality. Our clinical

research focuses on the relationship between systemic disorders such as life-style related diseases and aging, and bone disease. In basic research, we examine hard tissue properties in mucopolysaccharidosis-model mice and analyze epigenetic modifications of giant cell tumors. In the giant cell tumor cells we found a novel mutation in the H3.3 histone.

Publications

Yoshida M, Funasaki H, Marumo K. Efficacy of autologous leukocyte-reduced platelet-rich plasma therapy for patellar tendinopathy in a rat treadmill model. *Muscles Ligaments Tendons J.* 2016; **6:** 205-15.

Taguchi T, Kubota M, Saito M, Hattori H, Kimura T, Marumo K. Quantitative and qualitative change of collagen of achilles tendons in rats with systemic administration of glucocorticoids. *Foot Ankle Int.* 2016; **37:** 327-33.

Kayama T, Mori M[1], Ito Y[1], Matsushima T[1], Nakamichi R[1], Suzuki H[1], Ichinose S[1], Saito M, Marumo K, Asahara H[1] ([1]Tokyo Medical and Dental Univ). Gtf2ird1-dependent Mohawk (Mkx) expression regulates mechanosensing properties of the tendon. *Mol Cell Biol.* 2016; **36:** 1297-309.

Otani T, Futami T[1], Kita A[2], Kitano T[3], Saisu T[4], Satsuma S[5], Kawaguchi Y ([1]Shiga Medical Center for Children, [2]Japanese Red Cross Sendai Hosp, [3]Osaka City general Hosp, [4]Chiba Children's Hosp, [5]Kobe Children's Hosp). Treatment for unstable slipped capital femoral epiphysis: Current status and future challenge in Japan. *J Orthop Sci.* 2016; **21:** 847-51.

Shinohara A, Sairyo K[1], Mishiro T[2], Chikawa T[3], Soshi S ([1]Tokushima Univ, [2]Takamatsu Red Cross Hosp, [3]Tokushima Municipal Hosp). Insertional torque in cervical vertebrae lateral mass screw fixation: Magerl technique vs. Roy-Camille technique. *Clinical Spine Surg.* 2017; **30:** E1156-9. Epub 2016 Sep 10.

Ikeda R, Gu J[1] ([1]The Univ of Cincinnati College of Medicine). [EXPRESS] Electrophysiological property and chemical sensitivity of primary afferent neurons that innervate rat whisker hair follicles. *Mol Pain.* Epub 2016 Dec 7.

Chang W[1], Kanda H[1], Ikeda R, Ling J[1], DeBerry JJ[1], Gu JG[1] ([1]The Univ of Cincinnati College of Medicine). Merkel disc is a serotonergic synapse in the epidermis for transmitting tactile signals in mammals. *Proc Natl Acad Sci USA.* 2016 Sep 13; **113:** E5491-500.

Kondoh H[1], Nishiyama T[1], Kikuchi Y[2], Fukayama M[2], Saito M, Kii I[1], Kudo A[1] ([1]Tokyo Institute of Technology, [2]Tokyo Univ). Periostin deficiency causes severe and lethal lung injury in mice with bleomycin administration. *J Histochem Cytochem.* 2016; **64:** 441-53.

Miyasaka T, Kurosaka D, Saito M, Omori T, Ikeda R, Marumo K. Accuracy of computed tomography-based navigation-assisted total knee arthroplasty: outlier analysis. *J Arthroplasty.* 2017; **32:** 47-52.

Kimura T, Kubota M, Taguchi T , Suzuki N, Hattori A, Marumo K. Evaluation of first-ray mobility in patients with hallux valgus using weight-bearing CT and a 3-DAnalysis system. A comparison with normal feet. *J Bone Joint Surg Am.* 2017; **99:** 247-55.

Yamamoto S, Tanaka T, Sato K, Denda R, Kumagae Y, Sekiya H, Kojima M. Primary non-germal center B-cell-like diffuse large B-cell lymphoma arising from the patella. *J Orthopaedic Sci*. Epub 2017 Feb 13.

Ushiku C, Suda K[1], Matsumoto S[1], Komatsu M[1], Takahata M[1], Iwasaki N[1], Minami A[1] ([1]Hokkaido Spinal Cord Injury Center). Dural penetration caused by a vertebral bone fragment in a lumbar burst fracture: a case report. *Spinal Cord Ser Cases*. Epub 2017 Jan 12.

Kimura S, Saito M, Kida Y, Seki A, Isaka Y, Marumo K. Effects of raloxifene and alendronate on non-enzymatic collagen cross-links and bone strength in ovariectomized rabbits in sequential treatments after daily human parathyroid hormone (1-34) administration. *Osteoporos Int.* 2017; **28:** 1109-19.

Mashiba T[1], Saito M, Yamagami Y[1], Iwata K[1], Yamamoto T[1] ([1]Kagawa Univ Faculty of Medicine). Effects of suppressed bone remodeling by minodronic acid and alendronate on bone mass, microdamage accumulation, collagen crosslinks and bone mechanical properties in the lumbar vertebra of ovariectomized cynomolgus monkeys. *Bone.* 2017; **97:** 184-91. Epub 2017 Jan 7.

Chang W[1], Kanda H[1], Ikeda R, Ling J[1], Gu JG[1] ([1]The Univ of Cincinnati College of Medicine). Serotonergic transmission at Merkel discs: modulation by exogenously applied chemical messengers and involvement of Ih currents. *J Neurochem.* 2017; **141:** 565-76. Epub 2017 Mar 27.

Department of Neurosurgery

Yuichi Murayama, *Professor*
Takaaki Yanagisawa, *Professor*
Satoshi Ikeuchi, *Associate Professor*
Tatsuhiro Joki, *Associate Professor*
Hiroyuki Takao, *Associate professor*
Hiroyasu Nagashima, *Assistant Professor*
Yasuharu Akasaki, *Assistant Professor*
Toru Terao, *Assistant Professor*
Takao Arai, *Assistant Professor*
Yuichiro Nonaka, *Assistant Professor*
Hiroki Ohashi, *Assistant Professor*
Satoshi Tani, *Professor*
Hisashi Onoue, *Professor*
Yuzuru Hasegawa, *Associate Professor*
Toshihiro Ishibashi, *Associate Professor*
Nobuyoshi Kaito, *Assistant Professor*
Tosihide Tanaka, *Assistant Professor*
Akira Ishoshima, *Assistant Professor*
Ichiro Yuki, *Assistant Professor*
Hideki Arakawa, *Assistant Professor*
Koreaki Irie, *Assistant Professor*
Shougo Kaku, *Assistant Professor*

General Summary

The research studies in our department, examining such topics as syringomyelia, endovascular surgery, mechanism of head injury, and pediatric neurosurgery, made good progress during the past year. Research in these areas was performed to international standards. Clinical research on brain tumors, hypothalamic disorders, and spine and spinal cord diseases has also continued.

Research Activities

Cerebrovascular diseases · Endovascular surgeries

1. Analysis on the natural history of unruptured intracranial aneurysms.

Since 2003, more than 3,000 intracranial aneurysm patients have been visited our department. As a leading aneurysm treatment center in the world, we have placed a great value on a precise real-time data base of the aneurysms patients.

We focused on the analysis of 1) natural history of the unruptured aneurysms, 2) risk factors associated with the rupture of aneurysms, and 3) risk factors associated with treatment.

2. Analysis of fluid dynamics of human intracranial aneurysms using a computational fluid dynamics (CFD) models.

The main topics of our current study include 1) development of novel parameters, 2) elucidation of relationships between the hemodynamic patterns and rupture risk, 3) Development of a novel CFD software dedicated to the image workstation for angiographic equipment.

3. Development of a novel intracranal stent device for the treatment of brain aneurysms.

A novel intracranial stent device for the treatment of brain aneurysm is currently under development.

A preclinical animal study is ongoing. This project is supported by the Ministry of Economy, Trade and Industry under a research grant. We are now in the final stage of consecutive experiments, and the results will be reported to the Ministry of Economy, Trade and Industry in 2017.

4. Establishment of a tele-medicine network utilizing a novel software for smartphones.
Recently tele-medicine software “Join” became available for any smartphone users. The application allows every medical staff to have instant access to the PACS system of the Jikei university hospital, and allows each member to communicate using an online bulletin board system. The application is released in the collaboration with NTT Docomo, which is the Japan’s largest mobile service provider, serving more than 60 million customers

Brain tumor
1. Immunotherapy of malignant glioma
Effective antigen presentation to T cell subsets, such as CD8+ and CD4+ T cells, is a critical step in the generation and maintenance of immune responses against cancer cells. Although several cell types have the ability to present antigen, this function is performed most efficiently by professional antigen presenting cells, of which dendritic cells (DC) are the most potent. After exposure to tumor-associated antigens (TAA), DCs process and express TAA-derived epitopes in combination with MHC class I and II molecules on their cell surface, which induce TAA-specific cytotoxic T-lymphocyte (CTL) and T-helper type 1 (Th1) subsets, respectively. We had earlier shown that immunotherapy with fusion cells (FC) of DCs and glioma cells induces safe, tumor specific immune responses in glioma patients. In the recent study, we observed that Poly(I:C) transfected FCs induced high levels of endogenous IL-12 secretion from FCs. We also found that the ability of Poly(I:C)-transfected FCs to produce IL-12 was preserved when endogenous IL-10 was suppressed by small interference RNA (siRNA) of IL-10 (IL-10-siRNA) and that FCs cotransfected with IL-10-siRNA and Poly(I:C) elicited an efficient tumor-specific Th1 response. We started a clinical trial of “Immunotherapy with fusions of glioma cells, glioma initiating cells, and DCs ”.
2. Study of intra operative imaging using C-arm CT
We use a C-arm CT, syngo DynaCT system (SIEMENS), as well as an image analyzing soft wear for metal-artifact reduction in surgical resection of brain tumors. An intra operative imaging by this system improves the resection ratio of tumors, together with a surgical navigation system and a photo-dynamic diagnosis by 5-ALA. This study is aimed at optimizing safe technical innovations in surgery of brain tumors.

Neurotrauma
Few institutions have performed research in neurotraumatology. A unique aspect of our department is that we have undertaken 3 major studies in this area of research. We examined the prevalence of sports-related head injury in collaboration with the Japan Society of Clinical Sports Medicine and the Japan Society of Neurotraumatology. We have also examined sports-related concussion and performed mechanical studies of head injury through simulations.

Spine and Syringomyelia
About 50 patients with syringomyelia are treated surgically in our department each year. By evaluating cerebrospinal fluid (CSF) obstruction at the craniovertebral junction in

patients with syringomyelia related to Chiari malformation, the relation between CSF circulation blockage and cavitation of the spinal cord has been clarified. Therefore, improving the CSF circulation becomes the goal of surgical treatment. However, the mechanism of cavitation of the spinal cord itself is not fully understood. In patients with Chiari malformation, the cerebellar tonsils and the ventral vector (i.e., dens) compress the spinal cord and restrict CSF circulation. We examined whether these 2 factors influence the effects of foramen magnum decompression.

Division of Pediatric Neurosurgery

Division of pediatric neurosurgery conduct operations for patients with spina-bifida such as myeloschisis and spinal lipoma, hydrocephalus caused by various medical conditions, cranial facial anomaly, and brain tumor, etc., and follows them postoperatively at the outpatient clinic. There have been more than 1,700 new cases in various entities over the 10 years. We currently consist of a consultant, a division staff, and a resident, promoting clinical research through various clinical activities.

As for spina-bifida, we are currently examining the prognosis of neurological functions by operating under neuro-monitoring. We are also developing operative procedures for hydrocephalus using neuroendoscope, with the application of navigation systems.

Publications

Backes D, Rinkel GJE, Greving JP, Velthuis BK, Murayama Y, Takao H, Ishibashi T, Igase M, terBrugge KG, Agid R, Jääskeläinen JE, Lindgren AE, Koivisto T, Fraunberg M, Matsubara S, Moroi J, Wong GKC, Abrigo JM, Igase K, Matsumoto K, Wermer MJH, van Walderveen MAA, Algra A, Vergouwen MDI. ELAPSS score for prediction of risk of growth of unruptured intracranial aneurysms. *Neurology.* 2017; **88:** 1600-6. Epub 2017 Mar 31.

Boisserand LS, Kodama T, Papassin J, Auzely R, Moisan A, Rome C, Detante O. Biomaterial Applications in Cell-Based Therapy in Experimental Stroke. *Stem Cells Int.* 2016; **2016:** 6810562. Epub 2016 May 4. Review.

Akasaki Y, Kikuchi T, Homma S, Koido S, Ohkusa T, Tasaki T, Hayashi K, Komita H, Watanabe N, Suzuki Y, Yamamoto Y, Mori R, Arai T, Tanaka T, Joki T, Yanagisawa T, Murayama Y. Phase I/II trial of combination with temozolomide chemotherapy and immunotherapy with fusions of dendritic and glioma cells in patients with glioblastoma. *Cancer Immunol Immunother.* 2016; **65:** 1499-509.

Suzuki T, Takao H, Suzuki T, Kambayashi Y, Watanabe M, Sakamoto H, Kan I, Nishimura K, Kaku S, Ishibashi T, Ikeuchi S, Yamamoto M, Fujii Y, Murayama Y. Determining the presence of thin-walled regions at high-pressure areas in unruptured cerebral aneurysms by using computational fluid dynamics. *Neurosurgery.* 2016; **79:** 589-95.

Suzuki T, Takao H, Fujimura S, Dahmani C, Ishibashi T, Mamori H, Fukushima N, Yamamoto M, Murayama Y. Selection of helical braided flow diverter stents based on hemodynamic performance and mechanical properties. *J Neurointerv Surg.* 2017; **9:** 999-1005. Epub 2016 Sep 19.

Suzuki T, Takao H, Suzuki T, Suzuki T, Masuda S, Dahmani C, Watanabe M, Mamori H, Ishibashi T, Yamamoto H, Yamamoto M, Murayama Y. Variability of hemodynamic parameters using the common viscosity assumption in a computational fluid dynamics analysis of intracranial aneurysms. *Technol Health Care.* 2017; **25:** 37-47.

Kambayashi Y, Takao H, Shinohara K, Suzuki T, Takayama S, Fujimura S, Masuda S, Watanabe M, Suzuki T, Dahmani C, Ishibashi T, Yamamoto M, Murayama Y. Computational fluid dynamics analysis of tandem carotid artery stenosis: Invsetigation of neurological complications after carotid artery stenting. *Technology and Hearth Care.* 2016; **24:** 673-9.

Ishibashi T, Takao H, Suzuki T, Yuki I, Kaku S, Kan I, Nishimura K, Suzuki T, Watanabe M, Karagiozov K, Murayama Y. Tailor made shaping of microcatheters using three-dimensional Printed vessel models for endovascular coil embolization. *Computers in Biology and Medicine.* 2016; **77:** 59-63.

Department of Plastic and Reconstructive Surgery

Takeshi Miyawaki, *Professor*
Kunitoshi Ninomiya, *Associate Professor*
Katsuhiro Ishida, *Assistant Professor*
Shintaro Matsuura, *Associate Professor*
Kimihiro Nojima, *Associate Professor*

General Summary

Research in the Department of Plastic and Reconstructive Surgery is focused on 4 basic areas: 1) the causes and treatment of craniofacial anomalies, 2) the causes and treatment of hand and foot anomalies, 3) the mechanism of wound healing and grafting of the skin and bone, and 4) microsurgical transplantation. The faculty of our department consists of surgeons representing virtually all areas of plastic surgery and clinicians from related disciplines. This diversity provides the stimulating atmosphere necessary for productive research. The participation of plastic surgery residents and postresidency fellows in research studies provides them with important experience and expands their understanding of anatomical and physiological factors involved in these special areas of surgery.

Research Activities

Introducing the techniques of aesthetic surgery in open septorhinoplasty
Rhinoplasty plays a great role in the treatment of nasal obstruction, as the anterior nasal airway is responsible for 70% of airway resistance. Although caudal septal deviation is known to cause nasal obstruction, it has been untreated in the past in Japan as the caudal septum is a key structure to be preserved in conventional intranasal septoplasty. Damage to the caudal septum may compromise the shape of the nasal pyramid. Recently we have been collaborating with otorhinolaryngological surgeons in functional rhinoplasty and have introduced open septorhinoplasty techniques that are widely used in aesthetic surgery. The open approach allows correction of the deviated L-strut under direct vision, and also it is best indicated in the treatment of caudal septal deviation and internal/external nasal valve obstruction.

Treatment of nasal valve obstruction
The nasal valve region plays a key role in nasal breathing. Although a variety of techniques have been described to treat nasal valve compromise in the international literature, they are rarely used in Japan. Both nostrils collapsed completely during forced inspiration due to the weak cartilagenous support. There was no nasal deformity other than narrowing of both nostrils. Preoperative computed tomography revealed that the nasal septum was straight and the inferior turbinate was not swollen. Anterior nasomanometry showed that nasal resistance in the sitting position was increased preoperatively. Open septorhinoplasty was performed, and a 10-mm-wide L strut was left intact. The internal nasal valve was widened with a pair of spreader grafts. The external nasal valve was reinforced with the techniques of a columellar strut and an alar batten graft. The spreader graft was given the role of septal extension graft to support the tip of the nose. Postoperative nasal resis-

tance was less than the standard for adults, and the nostrils never collapsed during forced inspiration. Nasal valve compromise can cause nasal obstruction, even when the septum is straight, but it can easily be treated with well-known technique in aesthetic surgery.

Ilizarov Minifixator
The Ilizarov minifixator is a useful device in various areas of hand surgery. Its clinical usefulness was demonstrated in the treatment of fractures (open and comminuted fractures, fracture adjacent to the joint), malunion of fractures, pathological fractures caused by enchondroma and joint contractures, It was also used with good results in bone lengthening and the temporary traction of joints. Use of the Ilizarov minifixator is an effective yet noninvasive method and is highly recommended for selected cases.

Vascularized cutaneous perforator flap reconstruction after partial hypopharyngectomy with laryngeal preservation
Surgical resection of hypopharyngeal cancer often affects laryngeal functions. The aim of our study was to retrospectively assess the reliability and efficacy of vascularized cutaneous perforator flap transfer after partial hypopharyngectomy with laryngeal preservation. The subjects were 54 patients either underwent vascularized cutaneous perforator flap reconstruction immediately after partial pharyngolaryngectomy or hypopharyngectomy with laryngeal preservation. The defects were classified into 4 types on the basis of the area of the hypopharyngeal defect. Functional results were evaluated by means of routine physical examination, variables related to swallowing, and X-ray barium deglutition examination. Perioperative mortality and morbidity were reviewed. There were no perioperative deaths, and 98% of the flaps survived. Forty-three patients (80%) were able to eat an unrestricted diet and experienced no aspiration pneumonia. Restriction of the diet was significantly correlated with the extent of esophageal mucosal resection. Vascularized cutaneous perforator flap reconstruction is confirmed to be a safe and effective strategy for maintaining laryngeal function and good quality of life.

Assessment of surgical complications with the Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity in head and neck reconstruction
The usefulness of the Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity (POSSUM) for evaluating the risk of reconstructive surgery after the resection of head and neck cancers was examined. A total of 188 patients who underwent head and neck reconstruction after cancer extirpation from January 2010 through December 2011 were studied. The predicted risk of complications was calculated with the POSSUM and compared with the actual rate of perioperative complications. Perioperative complications occurred in 35 patients (19%), consisting of systemic complications in 17 patients (9%) and surgical site infection in 20 patients (11%). The patients were divided into a perioperative complication group and a noncomplication group. A significant difference between the groups was observed in terms of predicted postoperative rate calculated from the POSSUM ($p = 0.01$). The POSSUM is a useful indicator of the risk of reconstructive surgery after the resection of head and neck cancers. The cutoff value of the POSSUM calculated from the receiver operating characteristic curve using Youden's

index was 45.9%. Therefore, patients might be considered to be at high risk of perioperative complications when the POSSUM is 45.9% or greater.

Department of Cardiovascular Surgery

Kazuhiro Hashimoto, *Professor*
Ko Bando, *Professor*
Ryuichi Nagahori, *Associate Professor*
Yoshimasa Uno, *Asssitant Professor*
Hirokuni Naganuma, *Assistant Professor*
Yoshihiro Ko, *Assistant Professor*
Kiyozo Morita, *Professor*
Yoshimasa Sakamoto, *Associate Professor*
Koji Nomura, *Associate Professor*
Michio Yoshitake, *Assistant Professor*
Yoko Matsumura, *Assistant Professor*

General Summary

The major achievements in our department included both clinical studies and experimental animal studies. The clinical studies include those establishing excellent surgical performance, investigating new techniques, and evaluating alterations in cardiac performance and long-term results after cardiac surgery. In addition, analysis based on the JCVSD (big database for Japanese cardiac surgery) is becoming new projects. We are also continuously performing several experimental studies with in-vivo models. The major activities in adults and congenital sections are described below.

Research Activities

Echocardiographic Evaluation of postoperative coaptation geometry of Left Atrioventricular Valve (LAVV) in Complete Atrioventricular Septal Defect (cAVSD)
Postopertiev echocardiographic assessment was performed in 18 patients who underwent cAVSD repair to analyze LAVV function

Clinical investigation on myocardial protection during a pediatric heart surgery
In the infants who underwent open heart surgery for VSD, AVSD or the other congenital malformations with a various cardioplegic strategy, biochemical marker for myocardial injury (troponin T) and oxidative stress (8-iso-prostane) were evaluated.

Experimental studies of "remote per-conditioning" as a new therapeutic strategy of myocardial protection
An experimental study in an in-vivo piglet model was performed to test the hypothesis that ischemia/reperfusion (I/R)-induced biochemical damage and LV dysfunction can be reduced by "remote per-conditioning" (intermittent I/R of a remote organ before myocardial reperfusion).

Visualization of the cardiac conduction system in human heart specimens by the high-resolution phase contrast CT imaging
The visualization of the AV conduction axis within whole heart specimens was feasible with the use of a synchrotron radiation phase-contrast CT (PCCT) and verified by subsequent histological examination.

Clinical study of adult cardiac surgery

1. Choice of Aortic Valve Prosthesis in a Rapidly Aging and Long-living Society

Purpose: The aim of this study was to evaluate the long-term results of aortic valve replacement (AVR) with mechanical (M) and bioprosthetic (B) selected based on the current Japanese guidelines that recommend a B valve in patients aged more than 65 years. Methods: From April 1995 to March 2014, 366 adult patients underwent AVR or combined AVR/coronary artery bypass grafting. Of these, 127 (35%) patients received M valves and 239 patients (65%) received B valves. A retrospective analysis of the entire population and the selected 124 patients aged 60 to 70 years was carried out in order to compare the results between the two groups. Results: The 15-year overall survival was 87% ± 4% for the M group and 40% ± 29% for the B group. Freedom from reoperation at 15 years was 98% ± 2% for the M group and 82% ± 9% for the B group. Among propensity score matching of the subgroup in patients aged 60 to 70 years, there was also no significant difference in the 15-year survival and freedom from reoperation between the M and the B valves. Conclusion: The age criteria of 65 years for choosing an aortic bioprosthetic has been suitable.

2. Structural valve deterioration of a Carpentier-Edwards aortic pericardial bioprosthesis

In a recent aging society, patients who require valve operations have been getting old and the frequency of using mechanical valves has decreased extremely. That is because tissue valves have demonstrated satisfactory long-term durability and younger population who likes to live without anticoagulation has a tendency to increase despite a risk of reoperation due to structural valve deterioration (SVD). Moreover, the transcatheter valve-in-valve procedure has proven feasible for SVD as an alternative to surgical procedure. Although SVD is still an inevitable and most common cause for reoperation of tissue valves, it can be sometimes difficult to assess an appropriate timing of redo operation in asymptomatic young patients under carful echocardiographic follow-up. In any case, we have to follow the patients carefully so as not to lose an appropriate timing of a redo operation under stable condition and it is also important to monitor the outcomes of these patients who underwent bioprosthetic aortic replacement in young age.

3. Japanese Study of Bidirectional Evaluation of Surgical Performance on Cardiovascular Surgery (jBLADE Study-0)

Background: The cardiac surgery procedure consists of meticulous steps including: (1) opening the chest; (2) establishment of cardiopulmonary bypass (CPB); (3) harvesting saphenous vein graft; (4) harvesting the internal mammary artery for coronary artery bypass grafting (CABG), (5) main procedures, such as aortic valve replacement, mitral valve replacement, and mitral valvuloplasty; (6) cessation of CPB; and (7) closing the chest. Every trainee should become familiar with and, finally, gain expertise in each step of these procedures. scheduled to elucidate the logistics of the study and the standardized evaluation form.

Purpose: The purposes of the study were (1) to establish, objective, generalize, and standardize then evaluation system and (2) to elucidate the logistics of obtaining informed consent, evaluation of surgical performance, data acquisition, data transfer and management, and final analysis.

Method: Included in this study were board-eligible and board-certified trainees before

their first renewal who agreed to participate in the jBLADE-0 study.
Technical skills of these participants will be monitored with video recording. Video records of each case were blinded and evaluated by members of the evaluation committee. As a pilot study, 5 cases of each of 5 modules, including (1) opening chest, (2) establishment of CPB, (3) harvesting saphenous vein graft, (4) harvesting the IMA, and (5) closing chest in 6 institutions, were evaluated, and standardized evaluation criterion were established.
4. Japanese Study of Impact of Body Mass Index on Morbidity and Mortality in Geriatric Patients. Part 1: Coronary Artery Bypass Grafting
Objective: We sought to determine the effect of preoperative nutritional status determined by the body mass index on early mortality and morbidity after CABG in Japan.
Methods: We retrospectively identified 35,674 elderly patients (age ≥ 60 years) who had undergone CABG from January 1, 2008, to December 31, 2012, and had been registered in the Japanese Adult Cardiovascular Surgery Database. These patients were divided into 4 groups on the basis of body mass index. The primary endpoint was defined as early mortality, and the secondary endpoints were defined as composite endpoints, including stroke, transient ischemic attack, new dialysis, mediastinitis, and prolonged ventilation (≥ 24 hours).

Publications

Sakamoto Y, Yoshitake M, Matsumura Y, Naruse H, Bando K, Hashimoto K. Choice of Aortic Valve Prosthesis in a Rapidly Aging and Long-Living Society. *Ann Thorac Cardiovasc Surg.* 2016; **22:** 333-9.
Uno Y, Masuoka A, Hotoda K, Katogi T, Suzuki T. Evaluation of 60 cases of systemic-pulmonary shunt with cardiopulmonary bypass. *Gen Thorac Cardiovasc surg.* 2016; **64:** 592-6.
Shinohara G, Morita K, Hoshino M, Ko Y, Tsukube T, Kaneko Y, Morishita H, Oshima Y, Matsuhisa H, Iwaki R, Takahashi M, Matsuyama T, Hashinoto K, Yagi N. Three Dimensional Visualization of Human Cardiac Conduction Tissue in Whole Heart Specimens by High-Resolution Phase-Contrast CT Imaging Using Synchrotron Radiation. *World J Pediatr Congenit Heart Surg.* 2016; **7:** 700-5.
Nishioka N, Yamada K, Ujihira A, Iba Y, Maruyama R, Hatta E, Kurimoto Y, Nakanishi K. Outcomes of surgical aortic valve replacement using Carpentier-Edwards PERIMOUNT bioprosthesis series in elderly patients with severe aortic valve stenosis: a retrospective cohort study. *Gen Thorac Cardiovasc Surg.* 2016; **64:** 728-34.
Nishioka N, Kurimoto Y, Maruyama R, Ujihira K, Iba Y, Hatta E, Yamada A, Nakanishi K. Bladder and rectal incontinence without paraplegia or paraparesis after endova scular aneurysm repair. *Surg Case Rep.* 2016; **2:** 11.
Nakao M, Kawai Y, Niitsu H, Toyoda Y, Tsuda Y, Shiratori K, Takemura T, Bando K, Hashimoto K. Aortic root replacement for annuloaortic ectasia with a single coronary artery. *Gen Thorac Cardiovasc Surg.* 2016; **64:** 742-4.
Okita Y, Minakata K, Yasuno S, Uozumi R, Sato T, Ueshima K, Konishi H, Morita N, Harada M, Kobayashi J, Suehiro S, Kawahito K, Okabayashi H, Takanashi S, Ueda Y, Usui A, Imoto K, Tanaka H, Okamura Y, Sakata R, Yaku H, Tanemoto K, Imoto Y, Hashimoto K, Bando K. Optimal timing of surgery for active infective endocarditis with cerebral complications: a Japanese multicentre study. *Eur J Cardiothorac Surg.* 2016; **50:** 374-82.

Department of Obstetrics and Gynecology

Aikou Okamoto, *Professor*
Seiji Isonishi, *Professor*
Hirokuni Takano, *Associate Professor*
Osamu Samura, *Associate Professor*
Hiroshi Tanabe, *Assistant Professor*
Motoaki Saito, *Assistant Professor*
Kazuhiko Ochiai, *Professor*
Shigeki Niimi, *Professor*
Kyosuke Yamada, *Associate Professor*
Kouhei Sugimoto, *Assistant Professor*
Nozomu Yanaihara, *Assistant Professor*
Kazu Ueda, *Assistant Professor*

General Summary

The main research topics of our department are the development of molecularly targeted agents for gynecologic tumors, including ovarian cancer; clarification of the mechanisms of successful pregnancy; and the development of assisted reproductive techniques. These topics were investigated both experimentally and clinically.

Research Activities

Gynecologic oncology

1. Development of molecular targeting therapy in *ARID1A*-deficient cancers.

ARID1A, SWI/SNF chromatin remodeling complex subunit, has been identified as one of the most frequently mutated genes in human cancers. *ARID1A* mutations rates ranging from 10% to 57% have been identified across multiple tumor lineages, including ovarian clear cell carcinoma. In this study, we identified several compounds as a potential therapeutic targets for *ARID1A*-mutant cancers by screening *ARID1A* wild-type and *ARID1A* knockout cancer cells with an inhibitor kit. To explore the interaction between *ARID1A*-mutant cancers and the compound, we will conduct further assays.

2. Prognostic impact of interleukin-6 expression in stage I ovarian clear cell carcinoma.

In stage I ovarian clear cell carcinoma (OCCC), the prognosis differs according to substage, and predictive biomarkers are needed for stage IC2/IC3 disease. We investigated prognostic factors for stage I OCCC from a clinicopathological perspective with 192 patients, including the expression of ARID1A and IL-6. We calculated overall survival (OS) with respect to 12 clinicopathological parameters. The multivariate analysis indicated that substage classification and IL-6 expression status were associated with poor OS. Loss of ARID1A expression was related with substage, but not with survival. No clear link was found between ARID1A and IL-6 expression. Histological findings showed no prognostic effects. IL-6 molecular stratification may be crucial in optimizing therapeutic strategies for early stage OCCC to improve survival.

3. MicroRNA Gene Expression Signature Driven by *miR-9* Overexpression in Ovarian Clear Cell Carcinoma.

This study aimed to elucidate potential clinical and biological associations of ovarian cancer-related microRNA (miRNA) gene expression profiles in high-grade serous carcinoma and ovarian clear cell carcinoma (OCCC). Global cancer-related miRNA expression analysis identified statistically unique profiles that could discriminate ovarian cancer

histotypes. In OCCC, *miR-9* overexpression may affect pathogenesis by targeting E-cadherin, thereby inducing an epithelial-mesenchymal transition. Therefore, *miR-9* may be a promising therapeutic target strategy for OCCC.

4. Feasibility of reduced port surgery applying Higuchi's transverse incision.

Higuchi's transverse incision is made at a lower position than other incisions and is superior in terms of cosmetic outcomes. We examined the safety and efficacy of novel forms of reduced port surgery for ovarian cysts, patient characteristics and outcomes were compared between multiport laparoscopy and patients and uterine fibroids applying Higuchi's incision. In patients with ovarian cysts who underwent low-position single incision laparoscopic surgery (L-SILS) that was a modified single-port laparoscopy with Higuchi's incision placed in the 2-3cm just above the pubis. Additionally, patients with uterine fibroids who underwent dual-port laparoscopically assisted myomectomy (2P-LAM) and conventional LAM (C-LAM) were investigated. There were no significant differences between L-SILS and multiport laparoscopy in terms of fibroid size, bleeding, duration of hospital stay, or postoperative pain. However, L-SILS demonstrated significantly shorter operative and pneumoperitoneum times. In cases of fibroids, no significant differences were found in size, operative times, pneumoperitoneum times or bleeding. However, hospital stay of 2-P LAM group was shorter than C-LAM statistically. It was suggested that L-SILS for ovarian cysts and 2P-LAM for uterine fibroids are relatively simple and ensure the same safety and efficacy as conventional methods. Therefore, they have potential as novel forms of RPS.

Perinatology

1. Fetal therapy model of myelomeningocele with three-dimensional skin graft using amniotic fluid cell-derived induced pluripotent stem cells.

We generated induced pluripotent stem cells (iPSCs) from patients with Down syndrome and twin-twin transfusion syndrome. We manufactured three-dimensional skin grafts with epidermis generated from keratinocytes derived from iPSCs. For generation of epidermis, we developed a novel protocol using Y-27632 media and epidermal growth factor. The artificial skin was successfully covered over MMC defect sites during pregnancy, implying a possible antenatal surgical treatment utilizing iPSC technology.

2. Prenatal determination of fetal RHD focused on the difference of haplotype.

In Japanese, 80% of fetuses who are RHD negative, RHD loss is caused by RHD gene deletion, and 20% are caused by point mutation or hybrid RHD gene. Thus, many undiagnosed cases are occurred by conventional method. Our approach is focused on the difference of haplotype (single-nucleotide polymorphism combinations) between RHD positive and RHD negative to detect RHD negative specific haplotypes.

3. Elucidation of the role of oxytocin in perinatal brain.

Oxytocin has been reported to affect formation of analgesia, maternity and social behavior. Oxytocin not only induces labor, but also suppresses pain due to labor. We are investigating the effects of oxytocin on changes in neural tissue activity and influence on behavior using Ca-imaging to detect the activity of oxytocin (OXT) receptor in the amygdala using a rat model of the perinatal period.

4. Single cell DNA-sequence of fetal cells in maternal peripheral blood for noninvasive

prenatal diagnosis.
In order to analyze fetal genomic information safely and accurately, we are developing a new method for analyzing fetal DNA at the single cell level using purified circulating fetal cells in maternal peripheral blood. Our method can replace amniocentesis as a diagnostic antenatal test.
5. Investigation of Novel Candidate Genetic Factors Causing Recurrent Abortions in Japanese Women Using Whole-Genome Single Nucleotide Polymorphism Arrays.
High-resolution genome-wide single-nucleotide polymorphism microarray analysis were carried out among Japanese recurrent abortion cases with no obvious anatomical or medical causes. We attempt to analyze the genetic changes which have the potential to be the causative factors of recurrent abortions.
6. Genetic analysis for rare and undiagnosed cases.
Genomic and epigenetic analysis of patients with phenotypes unexplained by conventional chromosome examination may detect novel genetic and epigenetic aberrations. Detailed analysis including, copy number variations, exome analysis and methylation analysis are under examination to detect causative factors in rare cases.

Reproductive endocrinology
1. The utility and issues associated with the use of decision trees in oncofertility patient care.
To identify the utility and issues associated with the use of decision trees in oncofertility patient care, we investigated 35 women who had been diagnosed with cancer but had not begun anticancer treatment. We applied the oncofertility decision tree for women to counsel a consecutive series of women on fertility preservation (FP) options following cancer diagnosis. Oocyte retrieval was performed for 17 patients (48.6%; 36.35±3.82 years). The mean±SD number of cryopreserved embryos was 5.29±4.63. The expected live-birth rate was 0.66. In conclusions, the expected live-birth rate with FP indicated that 1 in 3 oncofertility patients would not expect to have a live birth following oocyte retrieval and embryo cryopreservation. While the decision trees were useful as decision-making tools for women contemplating FP, in the context of the current restrictions on oocyte donation and the extremely small number of adoptions in Japan, the remaining options for fertility after cancer are limited.
2. Study of Awareness of Adoption as a Family Building Option Among Oncofertility Stakeholders in Japan.
Adoption is an option to have a child for the survivor who lost their fertility due to oncologic treatment. However, it remains uncommon in Japan. We provided a questionary survey about adoption to Oncofertility stakeholders. Based on the reported answers, we concluded that doctors have insufficient knowledge about adoption, and survivors lack the self-confidence to even contemplate wanting children. On the other hand, adoption agencies are willing to consider cancer survivors as candidate adoptive parents on terms equal to those used for the general public provided the survivors meet parenting criteria. The present study demonstrated the need for healthcare professionals and reproductive specialists in particular to learn more about adoption and the importance of informing cancer survivors wishing to adopt that their medical history itself is not a hurdle.

Publications

Fukunaga M, Ishibashi T, Koyama T, Onoue K, Kitai S, Tanaka K, Isonishi S. Malignant Struma Ovarii With a Predominant Component of Anaplastic Carcinoma. *Int J Gynecol Pathol.* 2016; **35:** 357–61.

Seki T, Tanabe H, Nagata C[1], Suzuki J, Suzuki K, Takano H, Isonishi S, Ochiai K, Takakura S[2], Okamoto A ([1]National Center for Child Health and Development, [2]Dokkyo Med Univ Koshigaya Hosp). Adjuvant therapy after radical surgery for stage IB–IIB cervical adenocarcinoma with risk factors. *Jpn J Clin Oncol.* 2016; **47:** 32–8.

Yotsumoto J[1,2], Sekizawa A[2], Suzumori N[3], Yamada T[4], Samura O[5], Nishiyama M[5], Miura K[6], Sawai H[7], Murotsuki J[8], Kitagawa M[9], Kamei Y[10], Masuzaki H[6], Hirahara F[11], Endo T[12], Fukushima A[13], Namba A[10], Osada H[14], Kasai Y[15], Watanabe A[16], Katagiri Y[17], Takeshita N[17], Ogawa M[18], Okai T[19], Izumi S[20], Hamanoue H[11], Inuzuka M[1], Haino K[21], Hamajima N[22], Nishizawa H[23], Okamoto Y[24], Nakamura H[25], Kanegawa T[26], Yoshimatsu J[27], Tairaku S[28], Naruse K[29], Masuyama H[30], Hyodo M[31], Kaji T[32], Maeda K[33], Matsubara K[34], Ogawa M[35], Yoshizato T[36], Ohba T[37], Kawano Y[38], Sago H[5]; Japan NIPT Consortium ([1]Ochanomizu Univ, [2]Showa Univ Sch Med, [3]Nagoya City Univ Graduate Sch Medical Sciences, [4]Hokkaido Univ Graduate Sch Med, [5]National Center for Child Health and Development, [6]Nagasaki Univ, [7]Hyogo College Med, [8]Tohoku Univ Graduate Sch Med, Miyagi-Children's Hosp, [9]Sanno Hospital, [10]Saitama Medical Univ Hosp, [11]Yokohama City Univ Graduate Sch Med, [12]Sapporo Medical Univ Sch Med, [13]Department Clinical Genetics, Iwate Medical Univ Sch Med, [14]Chiba Univ Graduate Sch Med, [15]Jpn Red Cross Med Center, [16]Division Clinical Genetics, Nippon Medical Sch Hosp, [17]Toho Univ Omori Medical Center, [18]Perinatal Medical Center Women's Medical Univ Hosp, [19]Maternal and Child Health Center, Aiiku Hospital, [20]Tokai Univ Sch Med, [21]Niigata Univ Medical and Dental Hosp, [22]Dept Clin Genetics City West Med Center, [23]Fujita Health Univ, [24]Osaka Med Center and Research Institute for Maternal and Child Health, [25]Osaka City General Hosp, [26]Osaka Univ Faculty Med, [27]Department Perinatology and Gynecology, National Cerebral and Cardiovascular Center, [28]Kobe Univ Graduate Sch Med, [29]Nara Medical Univ, [30]Okayama Univ Graduate Sch Med, [31]Hiroshima Univ Graduate Sch Med, [32]The Univ Tokushima Faculty Med, [33]Shikoku Medical Center for Children and Adults, [34]Ehime Univ Sch Med, [35]Clinical Research Institute, National Hospital Organization Kyushu Medical Center, [36]Center for Maternal, Fetal and Neonatal Med, Fukuoka Univ Hosp, [37]Kumamoto Univ, [38]Oita Univ Hosp). A survey on awareness of genetic counseling for non-invasive prenatal testing: the first year experience in Japan. *J Hum Genet.* 2016; **61:** 995–1001.

Yanaihara N, Hirata Y, Yamaguchi N, Noguchi Y, Saito M, Nagata C[1] ([1]National Center for Child Health and Development), Takakura S, Yamada K, Okamoto A. Antitumor effects of interleukin-6 (IL-6)/interleukin-6 receptor (IL-6R) signaling pathway inhibition in clear cell carcinoma of the ovary. *Mol Carcinog.* 2016; **55:** 832–41.

Onda T[1], Satoh T[2], Saito T[3], Kasamatsu T[4], Nakanishi T[5], Nakamura K[6], Wakabayashi M[6], Takehara K[7], Saito M, Ushijima K[8], Kobayashi H[9], Kawana K[10], Yokota H[11], Takano M[12], Takeshima N[13], Watanabe Y[14], Yaegashi N[15], Konishi I[16], Kamura T[8], Yoshikawa H[2] ([1]Kitasato Univ, [2]Univ Tsukuba, [3]Natl Kyushu Cancer Center, [4]Natl Cancer Center Hosp, [5]Aichi Cancer Center Hosp, [6]JCOG Data Center, [7]Shikoku Cancer Center, [8]Kurume Univ, [9]Kyushu Univ, [10]Univ Tokyo Hosp, [11]Saitama Cancer Center, [12]Natl Defense Med Coll, [13]Cancer Institute Hosp Jpn Foundation for Cancer Research, [14]Kinki Univ, [15]Tohoku Univ, [16]Kyoto Univ). Comparison of treatment invasiveness between upfront debulking surgery versus interval debulking surgery following neoadjuvant chemotherapy for stage III/IV ovarian, tubal, and peritoneal cancers in a phase III randomised trial: Japan Clinical Oncology Group Study JCOG0602. *Eur J Cancer.* 2016; **64:** 22–31.

Ueda K, Nagayoshi Y, Kawabata A, Kuroda T, Iida Y, Saitou M, Yanaihara N, Sugimoto K, Sakamoto M, Okamoto A. Feasibility of reduced port surgery applying Higuchi's transverse incision. *Gynecology and Minimally Invasive Therapy*. 2016; **6:** 12–6.

Okamoto A, Nagayoshi Y, Kawabata A, Sakamoto M[1], Ueda K ([1]Kyoundo Hosp). Higuchi's transverse incision and a modification of this method for minimally invasive surgery. *Gynecology and Minimally Invasive Therapy*. 2016; **6:** 66–88.

Ueda K, Tabata J, Yanaihara N, Nagayoshi Y, Kawabata A, Onota S, Yamamoto R, Iida Y, Sugimoto K, Okamoto A. Laparoscopic conservative surgery for massive ovarian edema with torsion. *Gynecology and Minimally Invasive Therapy*. 2016; **5:** 91–3.

Yanaihara N, Noguchi Y, Saito M, Takenaka M, Takakura S[1], Yamada K, Okamoto A ([1]Dokkyo Med Univ Koshigaya Hosp). MicroRNA Gene Expression Signature Driven by miR-9 Overexpression in Ovarian Clear Cell Carcinoma. *PLoS One.* 2016; **11:** e0162584.

Kajiwara K, Tanemoto T, Nagata C, Okamoto A. Prenatal Diagnosis of Isolated Agnathia-Otocephaly: A Case Report and Review of the Literature. *Case Rep Obstet Gynecol.* 2016; 8512351.

Sugiyama T[1], Okamoto A, Enomoto T[2], Hamano T[3], Aotani E[4], Terao Y[5], Suzuki N[6], Mikami M[7], Yaegashi N[8], Kato K[9], Yoshikawa

***H*[10], *Yokoyama Y*[11], *Tanabe H, Nishino K*[12], *Nomura H*[13], *Kim JW*[14], *Kim BG*[15], *Pignata S*[16], *Alexandre J*[17], *Green J*[18], *Isonishi S, Terauchi F*[19], *Fujiwara K*[20], *Aoki D*[21] (*[1]Iwate Med Univ, [2]Niigata Univ, [3]Kitasato Univ, [4]Kanagawa Academy of Sci Technol, [5]Juntendo Univ, [6]St Marianna Univ, [7]Tokai Univ, [8]Tohoku Univ, [9]Kyushu Univ, [10]Univ Tsukuba, [11]Hirosaki Univ, [12]Niigata Univ, [13]Keio Univ, [14]Seoul Natl Univ, [15]Sungkyunkwan Univ, [16]Istituto Nazionale Tumori di Napoli, Naples, [17]Hôpital Hôtel-Dieu, [18]Univ Liverpool, [19]Tokyo Med Univ, [20]Saitama Med Univ International Med Center, [21]Keio Univ).** Randomized Phase III Trial of Irinotecan Plus Cisplatin Compared With Paclitaxel Plus Carboplatin As First-Line Chemotherapy for Ovarian Clear Cell Carcinoma. *Jpn J Clin Oncol.* 2016; **34:** 2881-7.

***Ito Y, Shiraishi E, Kato A, Haino T, Sugimoto K, Okamoto A, Suzuki N*[1] (*[1]St. Marianna Univ Sch Med).** The Utility of Decision Trees in Oncofertility Care in Japan. *J Adolesc Young Adult Oncol.* 2017; **6:** 186-9.

Department of Urology

Shin Egawa, *Professor*
Hiroshi Kiyota, *Professor*
Koji Asano, *Professor*
Takashi Hatano, *Assistant Professor*
Akira Furuta, *Assistant Professor*
Koichi Kishimoto, *Professor*
Nozomu Furuta, *Professor*
Yasuyuki Suzuki, *Associate Professor*
Kenta Miki, *Assistant Professor*
Takahiro Kimura, *Assistant Professor*

General Summary

We performed both basic and clinical research in the following areas: oncology, involving such sites as the kidney, bladder, prostate and testes; anatomy, physiology, and pharmacology of the bladder and urethra; imaging and radiology; infections and inflammation of the genitourinary tract, such as interstitial cystitis and prostatitis; infertility; andrology and sexual function; urolithiases; technology and instruments, such as laparoscopy; transplantation; neurourology; and female urology, such as benign prostatic hyperplasia, overactive bladder, neurogenic bladder, stress urinary incontinence, and pelvic floor prolapse.

Research Activities

1. Basic research: We performed several studies to elucidate the biology of urological malignancies, the mechanisms of voiding, and the pathophysiology of interstitial cystitis. Most studies have been presented at the annual meetings of the Japanese Urological Association and the American Urological Association. These projects are as follows;
1) Establishment and biological analysis of our new prostate cancer model, named JDCaP, derived from a Japanese patient.
2) Study of the incidence of latent prostate cancer.
3) Analysis of circulating tumor cells in castration-resistant prostate cancer.
4) Stem cell therapy for the treatment of interstitial cystitis, stress urinary incontinence and underactive bladder.
2. Clinical research: Several clinical studies have been investigated in our institution. The results of several studies have already been reported at the annual meetings of the Japanese Urological Association and the American Urological Association.
1) Prospective study of the efficacy of the sacral epidural block versus the pelvic plexus block for transrectal prostate needle biopsy.
2) Clinical study of sentinel lymph-node dissection for prostate and bladder cancer.
3) Analyses of urine markers including cytokines, chemokines and growth factors in patients with interstitial cystitis.

Publications

Hatano T, Chikaraishi K, Inaba H, Endo K, Egawa S. Outcomes of everolimus treatment for renal angiomyolipoma associated with tuberous sclerosis complex: a single institution experience in Japan. *Int J Urol.* 2016; **23:** 833-8.

Miki K, Sasaki H, Kido M, Takahashi H, Aoki M, Egawa S. A comparative study on the efficacies of gonadotropin-releasing hormone (GnRH)

agonist and GnRH antagonist in neoadjuvant androgen deprivation therapy combined with transperineal prostate brachytherapy for localized prostate cancer. *BMC cancer.* 2016 Sep 1; **16:** 708.

Furuta A, Suzuki Y, Kimura S, Koike Y, Egawa S, Yoshimura N. Combination therapy with β3-adrenoceptor agonists and muscarinic acetylcholine receptor antagonists : Efficacy in rats with bladder overactivity. *Int J Urol.* 2016; **23:** 425-30.

Furuta A. Editorial Comment to 5-HT2A receptor enhancement of contractile activity of the porcine urothelium and lamina propria. *Int J Urol.* 2016; **23:** 951.

Kimura T, Takahashi H, Okayasu M, Kido M, Inaba H, Kuruma H, Yamamoto T, Furusato B, Furusato M, Wada T, Egawa S. Time Trends in Histological Features of Latent Prostate Cancer in Japan. *J Urol.* 2016; **195:** 1415-20.

Kobayashi T, Kimura T, Lee C[1], Inoue T, Terada N, Kono Y, Kamba T, Kim CS[2], Egawa S, Ogawa O ([1]Department of Urology, Asan Medical Center, [2]Department of Urology, Asian Medical Center). Subclassification of high-risk clinically organ-confined prostate cancer for early cancer-specific mortality after radical prostatectomy. *Jpn J Clin Oncol.* 2016; **46:** 762-7.

Yoshimura K, Minami T, Nozawa M, Kimura T, Egawa S, Fujimoto H, Yamada A, Itoh K, Uemura H. A Phase 2 Randomized Controlled Trial of Personalized Peptide Vaccine Immunotherapy with Low-dose Dexamethasone Versus Dexamethasone Alone in Chemotherapy-naive Castration-resistant Prostate Cancer. *Eur Urol.* 2016; **70:** 35-41.

Kimura T, Egawa S. Re: Randomized Controlled Trial of Early Zoledronic Acid in Men with Castration-sensitive Prostate Cancer and Bone Metastases: Results of CALGB 90202 (Alliance). *Eur Urol.* 2016; **69:** 754-5.

Hata K, Kimura T, Tsuzuki S, Ishii G, Kido M, Yamamoto T, Sasaki H, Miki J, Yamada H, Furuta A, Miki K, Egawa S. Safety of fondaparinux for prevention of postoperative venous thromboembolism in urological malignancy: A prospective randomized clinical trial. *Int J Urol.* 2016; **23:** 923-8.

Hata K, Kimura T, Ishii G, Suzuki M, Egawa S. Non-immune mediated thrombocytopenia caused by thromboprophylaxis for the perioperative care of urological surgery: A case report and review of the literature. *Int J Surg.* 2016; **27:** 21-3.

Tabata R, Kimura T, Kuruma H, Sasaki H, Kido M, Miki K, Takahashi H, Aoki M, Egawa S. Do androgen deprivation and the biologically equivalent dose matter in low-dose-rate brachytherapy for intermediate-risk prostate cancer? *Cancer Med.* 2016; **5:** 2314-22.

Ishii G, Hata K, Aoki S, Suzuki M, Kimura T, Egawa S. Meningitis-retention Syndrome; A Case Report. *Urology.* 2016; **6:** 42-4.

Tsuzuki S, Sun Hee Park, Matthew R Eber, Christopher M Peters, Shiozawa Y. Skeletal complications in cancer patients with bone metastases. *Int J Urol.* 2016; **23:** 825-32.

Department of Ophthalmology

Tadashi Nakano, *Professor*
Hisato Gunji, *Professor*
Satoshi Nakadomari, *Associate Professor*
Akira Watanabe, *Associate Professor*
Takaaki Hayashi, *Associate Professor*
Koichi Kumegawa, *Associate Professor*
Yoshiaki Kabata, *Associate Professor*
Hirotsugu Takashina, *Associate Professor*
Hideo Kohno, *Associate Professor*
Shumpei Ogawa , *Associate Professor*
Keigo Shikishima, *Professor*
Genichiro Takahashi, *Associate Professor*
Masaki Yoshida, *Associate Professor*
Tsutomu Sakai, *Associate Professor*
Takuya Shiba, *Associate Professor*
Yoichiro Masuda, *Associate Professor*
Satoshi Goto, *Associate Professor*
Mikihide Ogasawara, *Associate Professor*
Hiroshi Horiguchi, *Associate Professor*

General Summary

The main research interest of our department is the pathophysiology of the visual processing system. The following topics are the subjects of basic and clinical studies: cataract, neuro-ophthalmology, ocular oncology and histopathology, biochemistry, functional magnetic resonance imaging (MRI), glaucoma, electrophysiology, diabetes, vitreoretinal diseases, age-related macular degeneration, uveitis, color vision, and the cornea.

Research Activities

Cataract
We are able to choose various premium intraocular lenses (IOLs), for example, multifocal IOLs, toric IOL, and yellow IOLs. We implant these new IOLs through microincisions and evaluate subsequent visual function.

Neuro-ophthalmology
1. Leber hereditary optic neuropathy (LHON) is a maternally inherited optic neuropathy that leads to central loss of vision, predominantly in young males. Most LHON cases have one of three primary point mutations in mitochondrial DNA (mtDNA). The annual incidence and prevalence of LHON in Japan are not known. Thus, we estimated the annual incidence of molecularly confirmed LHON in Japan during 2014. Sequential questionnaires were sent to 1,397 facilities and we received 861 responses to the first questionnaire. Approximately 120 cases (95% confidence interval ranged from 81 to 153) of newly developed LHON were reported during 2014 in Japan, and 93.2% were males. For the second questionnaire, responses were received from 30 facilities, and 86.5% of cases possessed the mtDNA ND4/G11778A mutation.
2. We report a rare case of macular hypoplasia with retinal folds in a patient with septo-optic dysplasia （SOD）. She had a history of hypoglycemia attacks and growth-hormone deficiency. Ophthalmoscopic examination revealed bilateral optic nerve hypoplasia and tortuous retinal vessels. Optical coherence tomography revealed foveal hypoplasia and retinal folds. Magnetic resonance imaging of the brain indicated atrophy of the bilateral optic nerves as well as atrophy of the optic chiasm and bilateral optic tracts. The pituitary

gland also exhibited atrophy. On the basis of genetic and environmental evidence, we hypothesize that a common factor causes both foveal hypoplasia and SOD.

Ocular oncology and histopathology

1. Immunoglobulin G4 (IgG4)-related disease is a novel clinical entity characterized by infiltration of IgG4-immunopositive plasmacytes and elevated serum IgG4 concentration accompanied by enlargement of and masses in various organs, including the lacrimal gland, salivary gland, and pancreas. Recent studies have clarified that conditions previously diagnosed as Mikulicz disease as well as various types of lymphoplasmacytic infiltrative disorders of the ocular adnexa are consistent with a diagnosis of IgG4-related disease. We review the new diagnostic criteria for IgG4-related ophthalmic disease, based on both the clinical and the histopathologic features of the ocular lesions.
2. We fully explained orbitotomy for anterior orbital tumors, clinical approach to orbital diseases, and examination for diagnosis and management of orbital diseases in textbooks.

Glaucoma

Analysis with the Markov model of the effects of an examination program showed that glaucoma produces an irreversible visual field loss and the most common type of visual impairment in Japan. Early detection and treatment are important until the advanced stage because symptoms are poor. We used the Markov model to analyze the effects of screening for glaucoma in adults. The early detection and early treatment of glaucoma are economically beneficial.

Functional neuroimaging

Cortical myelination was calculated with T1-weighted images divided by T2-weighted images as cortical myelin mapping with clinical MRI. In patients with hemianopsia and altered optic radiation, myelin content was reduced, particularly in the posterior portion of the primary visual cortex, but was better conserved in the anterior portion, respecting their visual field defects.

Developmental functional abnormality

Diffusion tensor imaging was performed to evaluate axonal-axonal density by means of fractional anisotropy on major white-matter tracts to compare subjects with and without strabismus. The fractional anisotropy value of the subjects with strabismus was reduced at the forceps major, which connects the occipital lobes via the splenium of corpus callosum.

Visual neuropsychology

With the use of functional MRI or diffusion MRI or both, many eye diseases have been shown to change the visual cortex and the visual tract. We are now attempting to stabilize a scanning procedure for quantitative MRI and to apply it to a volunteer who has an eye disease. Quantitative MRI allows us to directly measure T1 values. By using T1 values, we can estimate cell compositions at a voxel, each of which is an array of elements in a brain image.

Low vision

We assessed the effect of rehabilitation for patients with visual field loss by using the "Active Field Analyzer," which can be used to clarify a visual search function that is a factor in the specificity of the visual field but not in visual acuity.

vitreoretinal surgery

We have used 23-, 25-and 27-gauge transconjunctival vitrectomy system for macular hole, epiretinal membrane, macular edema and rhegmatogenous retinal detachment. The 25- and 23-gauge sutureless vitrectomy techniques decrease the surgical trauma and improve patients' postoperative comfort. The 25- and 23-gauge instrumentation is effective for a variety of vitreoretinal surgical indications. Although the infusion and aspiration rates of the 25- and 23-gauge instruments are lower than those for the 20-gauge high-speed vitrectomy system, the use of 25- and 23-gauge TVS may effectively reduce operative times of select cases that do not require the full capability of conventional vitrectomy.
To evaluate clinical efficacy of 7 mm intraocular lens (ETERNITY® Santen Pharmaceutical Co. Ltd.) for combined pars plana vitrectomy, phacoemulsification and intraocular lens implantation, we observed the visibility of the retina during vitrectomy and measured the depth of anterior chamber preoperatively and postoperatively with the PENTACAM®.
We are going to evaluate the changes in regular and irregular corneal astigmatism after 25-gauge and 23-gauge transconjunctival sutureless vitrectomy.
We investigated changes in corneal thickness following vitreous surgery and determined whether such changes can be used as a criterion for evaluating the invasiveness of vitrectomy.
As a method of treatment for a dropped lens nucleus that occurred during cataract surgery, we removed the dropped lens nucleus through the corneal wound without using a pars plana vitrectomy (PPV).

Electrophysiology

We are recording electroretinograms to evaluate whether there are functional disorders at the retinal-cell level in hereditary retinopathy, retinal dystrophy, and macular disease. The electroretinographic waveforms are compounded from the responses of various retinal cells, such as ganglion, amacrine, bipolar, and photoreceptor cells, which are recorded as a single wave pattern.

Diabetic Retinopathy section

A group of vulnerable retina ganglion cells has been reported in patients with diabetes mellitus and in animal models of diabetes. We are recording electroretinograms to evaluate retinal function in patients with diabetes but without retinopathy, as shown with ophthalmoscopy.

Uveitis

We reported on a patient with an atypical presentation of a phakic IOL who initially had vitelliform submaculopathy, a vitreous haze, and a peripheral retinal focus. We described detailed enface imaging of swept-source optical coherence tomography findings for 3

patients with acute zonal occult outer retinopathy.

Macular degeneration
We reported the effects of photodynamic therapy plus intravitreal aflibercept with subtenon triamcinolone acetonide injections for treating aflibercept-resistant polypoidal choroidal vasculopathy. Triple therapy improved visual and anatomical outcomes in patients who had PCV(Polypoidal choroidal vasculopathy) with recurrent or resistant retinal fluid and PED(pigment epithelial detachment) after multiple injections of intravitreal aflibercept.

Biochemistry
We examined the role of chemokines in a Abca4(−/−)Rdh8(−/−) mouse model of Stargardt disease and the Mertk(−/−) mouse model of retinitis pigmentosa. Our results indicated that the chemokine (C-C motif) ligand 3 gene (*Ccl3*) plays an essential role in regulating the severity of retinal inflammation and degeneration in these mouse models.

Color vision defects and genetic analysis of retinal diseases
1. Retinitis pigmentosa and its allied disorders have genetic heterogeneity. To identify pathogenic variants, we performed direct sequencing and whole-exome sequencing analysis for those disorders and successfully identified several novel pathogenic variants. In addition, among congenital color blindness, we analyzed genetic variations for congenital achromatopsia including congenital achromatopsia and blue cone monochromacy.

Cornea
We will assess the age and disease condition of patients with keratoconus and determine the most appropriate approach for improving vision and quality of life.

Publications

Nakano T, Kodaka F, Tsuneoka H. Differences in Neuroticism Between Patients with Glaucoma Who Have Discontinued Visits to Ophthalmologists and Those Who Make Regular Visits: Implications for Adherence to Topical Glaucoma Medications. *Ophthalmology and therapy.* 2016; **5:** 207-14.
Watanabe A, Tsuzuki A, Arai K, Gekka T, Tsuneoka H. Treatment of Dropped Nucleus with a 27-Gauge Twin Duty Cycle Vitreous Cutter. *Case Rep Ophthalmol.* 2016; **7:** 44-8.
Watanabe A, Tsuzuki A, Arai K, Gekka T, Kohzaki K, Tsuneoka H. Efficacy of Intravitreal Triamcinolone Acetonide for Diabetic Macular Edema After Vitrectomy. *J Ocul Pharmacol Ther.* 2016; **32:** 38-43.
Watanabe A, Gekka T, Arai K, Tsuneoka H. A case of traction retinal detachment in a patient with Gaucher disease. *Ophthalmic Genet.* 2016; 1-4.
Sakai T, Okano K, Kohno H, Tsuneoka H. Three-year visual outcomes of intravitreal ranibizumab with or without photodynamic therapy for polypoidal choroidal vasculopathy. *Acta Ophthalmol.* 2016; **94:** e765-71.
Higashi K, Meguro A, Takeuchi M, Yamane T, Kitaichi N, Horie Y, (Tsuneoka H), et al. Investigation of the association between IL10 gene polymorphisms and Vogt-Koyanagi-Harada disease in a Japanese population. *Ophthalmic Genet.* 2016; 1-3.
Hayashi T, Gekka T, Tsuneoka H. Spontaneous Resolution of Large Macular Retinoschisis in Enhanced S-Cone Syndrome. *Ophthalmic surgery, lasers & imaging retina*. 2016; **47:** 187-90.
Gocho K, Akeo K, Itoh N, Kameya S, Hayashi T, Katagiri S, Gekka T, Ohkuma Y, Tsuneoka H, Takahashi H. High-Resolution Adaptive Optics Retinal Image Analysis at Early Stage Central Areolar Choroidal Dystrophy With PRPH2 Mutation. *Ophthalmic surgery, lasers & imaging retina*. 2016; **47:** 1115-26.
Nakanishi A, Ueno S, Hayashi T, Katagiri S,

Kominami T, Ito Y, Gekka T, Masuda Y, Tsuneoka H, Shinoda K, Hirakata A, Inoue M, Fujinami K, Tsunoda K, Iwata T, Terasaki H. Clinical and genetic findings of autosomal recessive bestrophinopathy in Japanese cohort. *Am J Ophthalmol.* 2016 Aug; **168:** 86–94.

Kuniyoshi K, Hayashi T, Sakuramoto H, Mishima H, Tsuneoka H, Tsunoda K, Iwata T, Shimomura Y. New truncation mutation of the NR2E3 gene in a Japanese patient with enhanced S-cone syndrome. *Jpn J Ophthalmol.* 2016; **60:** 476–85.

Masuda Y, Iwaki H, Kato N, Takahashi G, Oki K, Tsuneoka H. Irrigation dynamic pressure-assisted hydrodissection during cataract surgery. *Clin Ophthalmol.* 2017; **11:** 323–8.

Masuda Y, Oki K, Iwaki H, Okamoto T, Tsuneoka H. Use of the phaco tip technique for lens cleavage and removal during cataract surgery. *Clin Ophthalmol.* 2016; **10:** 1925–9.

Takashina H, Watanabe A, Mitooka K, Tsuneoka H. Examination of Self-Sealing Sclerotomy for Vitrectomized Eye under Gas Tamponade in 23-Gauge Transconjunctival Sutureless Vitrectomy. *Semin Ophthalmol.* 2016; **31:** 210–4.

Takashina H, Watanabe A, Mitooka K, Tsuneoka H. Examination of Self-Sealing Sclerotomy for Vitrectomized Eye under Gas Tamponade in 23-Gauge Transconjunctival Sutureless Vitrectomy. *Semin Ophthalmol.* 2016; **31:** 210–4.

Ogawa T, Shiba T, Tsuneoka H. Usefulness of Surgical Media Center as a Cataract Surgery Educational Tool. *Journal of ophthalmology.* 2016; **2016:** 8435086.

Ogasawara M, Meguro A, Sakai T, Mizuki N, Takahashi T, Fujihara K, Tsuneoka H, Shikishima K. Genetic analysis of the aquaporin-4 gene for anti-AQP4 antibody-positive neuromyelitis optica in a Japanese population. *Jpn J Ophthalmol.* 2016; **60:** 198–205.

Kubota M, Shibata T, Gunji H, Tsuneoka H. Idiopathic horseshoe-like macular tear: a case report. *International medical case reports journal*. 2016; **9:** 219–22.

Iida K, Ohkuma Y, Hayashi T, Katagiri S, Fujita T, Tsunoda K, Yamada H, Tsuneoka H. A novel heterozygous splice site OPA1 mutation causes exon 10 skipping in Japanese patients with dominant optic atrophy. *Ophthalmic Genet.* 2016 Sep; **37:** 354–6.

Katagiri S, Hayashi T, Yoshitake K, Akahori M, Ikeo K, Gekka T, Tsuneoka H, Iwata T. Novel C8orf37 Mutations in Patients with Early-onset Retinal Dystrophy, Macular Atrophy, Cataracts, and High Myopia. *Ophthalmic Genet.* 2016; **37:** 68–75.

Katagiri S, Hayashi T, Kondo M, Tsukitome H, Yoshitake K, Akahori M, Ikeo K, Tsuneoka H, Iwata T. RPE65 Mutations in Two Japanese Families with Leber Congenital Amaurosis. *Ophthalmic Genet.* 2016; **37:** 161–9.

Katagiri S, Hayashi T, Gekka T, Tsuneoka H. A novel homozygous CYP4V2 variant (p.S121Y) associated with a choroideremia-like phenotype. *Ophthalmic Genet.* 2016; 1–2.

Ishikawa K, Gekka T, Hayashi T, Kikuchi S, Kameya S, Tsuneoka H. Closure of a full-thickness macular hole without vitrectomy in choroideraemia. *Clin Exp Optom.* 2016.

Komatsu K, Sakai T, Kaburaki T, Tsuji H, Tsuneoka H. Atypical presentation of primary intraocular lymphoma. *BMC Ophthalmol.* 2016; **16:** 171.

Department of Otorhinolaryngology

Hiromi Kojima, *Professor*
Yutaka Yamamoto, *Associate Professor*
Makoto Iida, *Associate Professor*
Daiya Asaka, *Assistant Professor*
Jiro Iimura, *Assistant Professor*
Takanori Hama, *Assistant Professor*
Nobuyoshi Otori, *Professor*
Atsushi Hatano, *Associate Professor*
Yoichi Seino, *Associate Professor*
Satoshi Chikazawa, *Assistant Professor*
Tomomi Fukuda, *Assistant Professor*

Research Activities

Research issues in otology

Our research projects span experiments on the fundamental aspects of middle ear mucosa regeneration and its clinical application, research on gene therapy targeting epithelium with residual cholesteatoma, and the development of a navigation system utilizing virtual-reality technology to increase the safety of surgery. In addition, cases of cholesteatoma surgery performed at our hospital are recorded in our database, which is used to analyze the condition of patients, to select operative methods, and to review postoperative outcomes. We perform approximately 250 middle ear surgeries annually at our hospital. Cochlear implantations performed every year have also yielded favorable results. We perform skull-base surgery, including that for cholesteatoma in the petrous part of the temporal bone, in conjunction with the Department of Neurosurgery, and have found that hearing and facial nerve function can be preserved in many cases. We also perform acoustic tumor surgery.

For secretory otitis media we select the treatment method in individual patients depending on the degree of development of the mastoid air cells.

In the field of neuro-otology, we have introduced vestibular evoked myogenic potential (VEMP) testing to evaluate saccular function in patients with such conditions as vestibular neuritis, Meniere's disease, and dizziness of unknown cause to facilitate diagnosis and treatment. Moreover, we are examining the prevalence of abnormal saccules in various disorders as measured with VEMP testing, the ictal and nonictal phases of Meniere's disease, and the incidence of VEMP abnormalities according to disease stage.

Research in rhinology

We are involved in the analysis of data on factors related to the intractability of rhinosinusitis obtained from patients undergoing endoscopic sinus surgery (ESS) and from prospective studies of the postoperative course. We perform special care for skull base diseases, such as pituitary tumors and CSF leak, with a good relationship with the Department of Neurosurgery. We report case studies and investigate the postoperative course of skull base diseases. In an attempt to expand the indications for ESS from paranasal sinus tumors to skull-base surgery, including that for spinal fluid leakage, skull-base tumors, and pituitary gland tumors, and to improve the safety of ESS, we have performed high-tech navigation surgery in which 3-dimensional endoscopic images and stereonavigation images are superimposed.

We have planned clinical studies and developed treatment methods for patients with a variety of olfactory disorders. We began rehabilitation for olfactory disorders for the first time in Japan. Since last year we have offered anatomy training using fresh-frozen cadavers at the Skills Laboratory, for both skull-base surgery and endoscopic sinus surgery training. We must improve both medical techniques and anatomical knowledge. To elucidate the pathogenesis of eosinophilic chronic rhinosinusitis and allergic fungal rhinosinusitis, we investigate how environment fungi and bacteria induce activation and degranulation of human eosinophils and the airway epithelium.

Research of head and neck tumors

For common advanced cancers we perform radical surgery (e.g., total pharyngolaryngectomy combined with reconstruction by means of free intestinal flap transfer for hypopharyngeal cancer and total laryngectomy for laryngeal cancer); however, we perform larynx-preserving surgery (partial hypopharyngectomy combined with reconstruction by means of free-flap transfer and partial laryngectomy) to preserve function, especially vocal function, to the greatest extent possible. We have obtained favorable outcomes in terms of both laryngeal preservation and survival. For conservative therapy and postoperative treatment for advanced cancer, we perform radiotherapy, alone or with concurrent chemotherapy with cisplatin and fluorouracil, and have obtained favorable results. We use narrow-band imaging endoscopy for diagnosis in routine practice and make good use of this technology for the diagnosis and treatment of early-stage superficial mesopharyngeal and hypopharyngeal cancers.

In regard to research on cancer, we are performing basic studies and applying their findings to future studies and to clinical practice; such fundamental studies include extraction of DNA from specimens obtained during surgery and evaluation of epidermal growth factor receptor expression, a target for molecularly targeted agents, such as the expression of human papilloma virus, which has been implicated in the development of mesopharyngeal cancer and oral cancer.

Research on vocal and swallowing functions

Phonosurgery: We are performing outpatient day surgery using a flexible fiberoptic laryngoscope and performing laryngomicrosurgery using the microflap method under general anesthesia for vocal fold polyps, vocal cord nodules, and vocal cord cysts. For many years we have performed injection of atelocollagen into the vocal folds as outpatient day surgery for unilateral recurrent nerve paralysis; however, we are also performing laryngeal framework surgery for patients who are considered poor candidates for atelocollagen injection.

Diagnosis and treatment of spasmodic dysphonia: Since December 2004 we have performed botulinum toxin treatment as a first-line therapy for spasmodic dysphonia with the approval of the ethics committee of the university. The prevalence of this disorder has been increasing; therefore, evaluating methods for diagnosis and treatment is of clinical importance.

Research on sleep apnea syndrome

To verify whether allergic rhinitis is involved in sleep disorders, research for patients with pollinosis has been performed since last year at the Ota Sleep Science Center.

Continuous positive airway pressure treatment will be the first choice for patients with obstructive sleep apnea syndrome of greater than moderate severity. On the other hand, the effectiveness and safety of surgical treatment are still unknown. We will be able to present the adaptation of surgical treatment for sleep disorders. Long-distance sleep examinations have been performed since 2009 at the Ota Sleep Science Center.

Publications

Morita Y, Yamamoto Y, Ohshima S, Takahashi K, Takahashi S. Pediatric middle ear cholesteatoma: the comparative study of congenital cholesteatoma and acquired cholesteatoma. *Eur Arch Otorhinolaryngol.* 2016; **273:** 1155-60.

Tono T, Sakagami M, Kojima H, Yamamoto Y, Matsuda K, Komori M, Hato N, Morita Y, Hashimoto S. Staging and classification criteria for middle ear cholesteatoma proposed by the Japan Otological Society. *Auris Nasus Larynx.* 2017 Apr; **44:** 135-40. Epub 2016 Sep 9.

Yamamoto-fukuda T, Takahashi H, Kojima H. KGF controls on the epithelial stem/progenitor cell proliferation in external auditory canal. *J Laryngol Otology.* 2016; **130:** 247.

Yamamoto K, Yamato M, Morino T, Sugiyama H, Takagi R, Yaguchi Y, Okano T, Kojima K. Middle ear mucosal regeneration by tissue-engineered cell sheet transplantation. *NPJ Regenerative Medicine.* 2017; **2:** 6. Epub 2017 Mar 24.

Nakayama T, Asaka D, Kanaya H, Kuboki A, Haruna S. Prognostic factors for recurrence after endoscopic sinus surgery for chronic rhinosinusitis with nasal polyps. *Auris Nasus Larynx.* 2016; **43:** 641-7.

Ando Y, Chiba S, Capasso R, Okushi T, Kojima H, Otori N, Wada K. Risk factors for sleep impairment in adult patients with chronic rhinosinusitis. *Auris Nasus Larynx.* 2016; **43:** 418-21.

Komori M, Yamamoto Y, Yaguchi Y, Ikezono T, Kojima H. Cochlin-tomoprotein test and hearing outcomes in surgically treated true idiopathic perilymph fistula. *Acta Otolaryngol.* 2016; **136:** 901-4.

Kobayashi T, Mizuta M, Hiwatashi N, Kishimoto Y, Nakamura T, Kanemaru S, Hirano S. Drug delivery system of basic fibroblast growth factor using gelatin hydrogel for restoration of acute vocal fold scar. *Auris Nasus Larynx.* 2017; **44:** 86-92.

Shimizu Y, Mori E, Wada K, Otori N, Kojima H. Airway intervention in cases of acute epiglottitis. *B-ENT.* 2016; **12:** 279-84.

Department of Anesthesiology

Shoichi Uezono, *Professor and Chairperson*
Naohito Shimoyama, *Professor*
Megumi Shimoyama, *Professor*
Akihiro Suzuki, *Professor*
Masaki Kitahara, *Associate Professor*
Ichiro Kondo, *Associate Professor*
Shigehiko Uchino, *Associate Professor*
Hiroshi Sunaga, *Associate Professor*
Keiko Kojima, *Assistant Professor*
Yukino Kubota, *Assistant Professor*
Kotaro Kida, *Assistant Professor*
Haściłowicz Tomasz, *Assistant Professor*
Sachiko Omi, *Professor*
Shuya Kiyama, *Professor*
Tsunehisa Tsubokawa, *Professor*
Masanori Takinami, *Associate Professor*
Chieko Fujiwara, *Associate Professor*
Yasushi Mio, *Associate Professor*
Yoichi Kase, *Associate Professor*
Kazuhiro Shoji, *Assistant Professor*
Gumi Hidano, *Assistant Professor*
Takako Terui, *Assistant Professor*
Kentaro Yamakawa, *Assistant Professor*

General Summary

The functions of the Department of Anesthesiology are to provide quality patient care, to teach, and to perform research in perioperative medicine, intensive care medicine, and comprehensive pain management. In 2016 we made further advances and great achievements with the support of our faculty, institutional administration, and the Dean of The Jikei University. Below we highlight some of our research achievements in 2016.

Research Activities

Research continues as a growing and important component of the department's activities. The department is committed to enhancing academic productivity and resources by dedicating time to research and granting clinical access to research cases.

The investigators have been successful each year in obtaining peer-reviewed research grants. For one thing, Grants-in-Aid for Scientific Research (*kakenhi*) were awarded to six members of our Faculty in 2016. The department continues to build on the strengths of several outstanding programs: cardiovascular anesthesia, thoracic anesthesia, pediatric anesthesia, regional anesthesia, neuroanesthesia, obstetric anesthesia, intensive care medicine, and comprehensive pain management. Faculty recruitment is targeted at individuals with demonstrated academic and research activities as well as excellent clinical management and teaching skills. In 2015 Dr. Suzuki was recruited to improve our educational programs of sonography. In 2016 he implemented new programs for our residents and junior Faculty to improve the quality of diagnostic procedures in acute care settings.

Our faculty and residents were both well represented at the Japanese Society of Anesthesiologists' annual meeting in Fukuoka and the American Society of Anesthesiologists' annual meeting in Chicago. In addition, members of the department continue to be invited as visiting professors or guest speakers at national and international meetings.

Listed below are some of the ongoing research projects in which the principal investigators are faculty members of the Department of Anesthesiology.

Doctors Uezono and Kida have been investigating the protective effects of sedatives in ischemic encephalopathy. Doctor Shimoyama's research has been focused on the mecha-

nism of mitochondria dysfunction-induced peripheral nerve injury. She also has been working to elucidate the mechanism of chemical induced neuropathic pain, which may lead to new therapeutic interventions for this type of pain.

In clinical medicine, several principal investigators from the Department of Anesthesiology deserve mention. Doctor Kondo has been interested in the concept of goal directed therapy and its application to fluid management during surgery for cancers of the head and neck. Doctor Uchino continues to be active in clinical research in the intensive care unit and has been extremely productive in the field of acute kidney injury. Using a large database in the intensive care unit, Drs. Uchino and Yoshida have been attempting to identify predictive values of new onset of atrial fibrillation after noncardiac surgeryies. Our pain clinic physicians led by Dr. Kitahara continue to play a pivotal role in establishing practice guidelines for patients with various types of chronic pain. One of their targets is postmastectomy pain.

The appended bibliography of the department shows that a wide range of investigative and scholarly activities were conducted over the past year.

Publications

Eto K, Kondo I, Kosuge M, Ohkuma M, Haruki K, Neki K, Sugano H, Hashizume R, Yanaga K. Enhanced Recovery After Surgery Programs for Laparoscopic Colorectal Resection May Not Need Thoracic Epidural Analgesia. *Anticancer Res.* 2017; **37:** 1359-64.

Sunaga H, Savarese JJ[1], McGilvra JD[2], Heerdt PM[1], Belmont MR[1], Van Ornum SG[3], Murrell MT[1], Malhotra JK[1], Savard PM[1], Jeannotte E[4], Petty BJ[1], Allen E[5], Carnathan GW[5] ([1]Weill Med Coll Cornell Univ, [2]Cedarburg Pharmaceuticals, [3]Concordia Univ, [4]Albany Medical Center, [5]Impact Pharmaceutical Services, Inc). Preclinical Pharmacology of CW002: A Nondepolarizing Neuromuscular Blocking Drug of Intermediate Duration, Degraded and Antagonized by l-cysteine-Additional Studies of Safety and Efficacy in the Anesthetized Rhesus Monkey and Cat. *Anesthesiology.* 2016; **125:** 732-43.

Heerdt PM[1], Sunaga H, Owen JS[2], Murrell MT[3], Malhotra JK[3], Godfrey D[3], Steinkamp M[3], Savard P[3], Savarese JJ[3], Lien CA[3] ([1]Yale Med Sch, [2]Union Univ, [3]Weill Cornell Medical Coll). Dose-response and Cardiopulmonary Side Effects of the Novel Neuromuscular-blocking Drug CW002 in Man. *Anesthesiology.* 2016; **125:** 1136-43.

Howard-Quijano K[1], McCabe M[1], Cheng A[1], Zhou W[1], Yamakawa K, Mazor E[1], Scovotti JC[1], Mahajan A[1] ([1]UCLA). Left ventricular endocardial and epicardial strain changes with apical myocardial ischemia in an open-chest porcine model. *Physiol Rep.* 2016 Dec; **4:** e13042.

Irie T[1]*, Yamakawa K*, Hamon D[2], Nakamura K[3], Shivkumar K[2], Vaseghi M[2] ([1]Gunma Univ, [2]UCLA, [3]Toho Univ). Cardiac Sympathetic Innervation Via the Middle Cervical and Stellate Ganglia and Anti-Arrhythmic Mechanism of Bilateral Stellectomy. *Am J Physiol Heart Circ Physiol.* 2017 Mar 1; **312:** H392-405.
*These authors contributed equally.

Howard-Quijano K[1], Takamiya T, Dale EA[1], Yamakawa K, Zhou W[1], Buckley U[1], Mahajan A[1] ([1]UCLA). Effect of Thoracic Epidural Anesthesia on Ventricular Excitability in a Porcine Model. *Anesthesiology.* 2017 Jun; **126:** 1096-106. Epub 2017 Mar 30.

Ikeda K, Liu X[1], Kida K, Marutani E[2], Hirai S[2], Sakaguchi M[2], Andersen LW[1], Bagchi A[2], Cocchi MN[1], Berg KM[1], Ichinose F[2], Donnino MW[1] ([1]Beth Israel Deaconess Medical Center, [2]Massachusetts General Hospital). Thiamine as a neuroprotective agent after cardiac arrest. *Resuscitation.* 2016; **105:** 138-44.

Miyazaki Y, Sunaga H, Hobo S, Miyano K, Uezono S. Pancuronium enhances isoflurane anesthesia in rats via inhibition of cerebral nicotinic acetylcholine receptors. *J Anesth.* 2016; **30:** 671-6.

Izawa J, Uchino S, Takinami M. A detailed evaluation of the new acute kidney injury criteria by KDIGO in critically ill patients. *J Anesth.* 2016 Apr; **30:** 215-22.

Katayama S (Asahi General Hospital), Uchino S, Uji M (Osaka University Hospital), Ohnuma T (Saitama Medical Center, Jichi Medical University), Namba Y (Showa University Fujigaoka Hospital), Kawarazaki H (Marianna University School of Medicine), Toki N (Tokyo Metropolitan Tama Medical Center), Takeda K (Hyogo College of Medicine), Yasuda H (Japanese Red Cross Musashino Hospital), Izawa J, Tokuhira N (Kyoto Prefectural University of Medicine), Nagata I (Kanto Rosai

Hospital). Factors predicting successful discontinuation of continuous renal replacement therapy. *Anaesth Intensive Care.* 2016 Jul; **44:** 453–7.

Izawa J, Kitamura T (Graduate School of Medicine, Osaka University), Iwami T (Kyoto University School of Public Health), Uchino S, Takinami M, Kellum JA (University of Pittsburgh Medical Center), Kawamura T (Kyoto University School of Public Health). Early-phase cumulative hypotension duration and severe-stage progression in oliguric acute kidney injury with and without sepsis: an observational study. *Crit Care.* 2016 Dec; **20:** 405.

Ohnuma T (Saitama Medical Center), Uchino S. Prediction Models and Their External Validation Studies for Mortality of Patients with Acute Kidney Injury: A Systematic Review. *PLoS One.* 2017 Jan 5; **12:** e0169341.

Fukushima T, Uchino S, Fujii T, Takinami M, Uezono S. Intraoperative hydroxyethyl starch 70/0.5 administration may increase postoperative bleeding: a retrospective cohort study. *J Anesth.* 2017 Mar 24. [Epub ahead of print].

Toyama S (National Center for Child Health and Development), Shimoyama N, Shimoyama M. The analgesic effect of orexin-A in a murine model of chemotherapy-induced neuropathic pain. *Neuropeptides.* 2017; **61:** 95–100.

Reviews and Books

Sunaga H, Blasberg JD[1], Heerdt PM[1] ([1]Yale School of Medicine). Anesthesia for nonintubated video-assisted thoracic surgery. *Curr Opin Anaesthesiol.* 2017; **30:** 1–6.

Kawarazaki H (Marianna University School of Medicine), Uchino S. Validity of low-efficacy continuous renal replacement therapy in critically ill patients. *Anaesthesiol Intensive Ther.* 2016; **48:** 191–6.

Department of Rehabilitation Medicine

Masahiro Abo, *Professor and Chairperson*
Kazushige Kobayashi, *Professor*
Itaru Takehara, *Associate Professor*
Hidekazu Sugawara, *Assistant Professor*
Tadashi Suzuki, *Assistant Professor*
Toru Takekawa, *Assistant Professor*
Ryo Momosaki, *Assistant Professor*
Shu Watanabe, *Professor*
Wataru Kakuda, *Associate Professor*
Kun Suk Chung, *Assistant Professor*
Masanori Funakoshi, *Assistant Professor*
Nobuyuki Sasaki, *Assistant Professor*
Anri Kamide, *Assistant Professor*

General Summary

The main research topics of our department are as follows: 1) effect of repetitive transcranial magnetic stimulation (rTMS), 2) dysphagia, 3) treatment for stroke, 4) analysis based on database.

Research Activities

Effect of rTMS

1. Combination protocol of low-frequency rTMS and intensive occupational therapy (iOT) for post-stroke upper limb hemiparesis: a 6-year experience of more than 1,700 Japanese patients

Our proposed combination protocol of rTMS and iOT for upper limb hemiparesis was proved a safe and useful therapeutic intervention by a result of a multi-institutional study on a total of 1,725 post-stroke patients.

2. Does a combined intervention program of rTMS and iOT affect cognitive function in patients with post-stroke upper limb hemiparesis?

We retrospectively investigated whether the combined treatment of rTMS and iOT influenced patient's cognitive function. Twenty-five patients received the treatment. Only patients with right-sided hemiparesis exhibited improved Trail-Making Test part B performance.

3. The effect of selective rTMS with functional near-infrared spectroscopy (fNIRS) and intensive speech therapy (iST) on individuals with post-stroke aphasia

The administration of fNIRS-guided selective rTMS therapy and iST to eight right-handed post-stroke patients with aphasia induced a significant improvement in language function.

4. High-frequency rTMS on leg motor area in the early phase of stroke

Twenty-one patients with a hemispheric stroke lesion in the early phase were randomly assigned into two groups: the high-frequency (HF)-rTMS group and the sham stimulation group. The improvement in Brunnstrom Recovery Stages for the lower limbs was significant after the intervention in the HF-rTMS group.

5. High-frequency rTMS for the treatment of chronic fatigue syndrome: a case series

We consecutively applied facilitatory high-frequency rTMS to the dorsolateral prefrontal cortex of seven chronic fatigue syndrome patients over three days. In most of the patients,

treatment resulted in an improvement of fatigue symptoms.

Dysphagia

1. Influence of repetitive peripheral magnetic stimulation on neural plasticity in the motor cortex related to swallowing

The results indicated that repetitive peripheral magnetic stimulation increased motor-evoked potential amplitude of swallowing muscles, suggesting facilitation of the motor cortex related to swallowing in healthy individuals.

2. Noninvasive brain stimulation for dysphagia after acquired brain injury: a systematic review

The review provided low-quality evidence for the effectiveness of noninvasive brain stimulation including rTMS and transcranial direct current stimulation in improving dysphagia after acquired brain injury.

Treatment for stroke

1. Atomoxetine administration combined with iST for post-stroke aphasia: evaluation by a novel SPECT method

Atomoxetine administration combined with iST were safe and feasible for post-stroke aphasia. Four patients showed improved language function and their cortical blood flow surrounding lesioned brain areas was found to increase following intervention.

2. Effect of home-based training using a slant board with dorsiflexed ankles on walking function in post-stroke hemiparetic patients

This home-based rehabilitation program using the slant board was safe and improved walking function in post-stroke hemiparetic patients. Six patients showed increased walking velocity, decreased the number of steps in the 10-m walking test, and decreased Timed “Up and Go” test performance time.

Analysis based on database

1. Predictive factors for oral intake after aspiration pneumonia in older adults

We clarified prognostic factors for total oral intake in elderly aspiration pneumonia patients. Early initiation of total oral intake was associated with female sex and higher Barthel Index. Delayed initiation of total oral intake was associated with underweight, higher scores of pneumonia severity and comorbidities.

2. Proton pump inhibitors (PPIs) versus histamine-2 receptor antagonists (H2RAs) and risk of pneumonia in patients with acute stroke

These data demonstrated that no significant difference in the incidence of pneumonia was seen between users of PPIs and H2RAs after acute stroke.

3. Impact of board-certificated physiatrists on rehabilitation outcomes in elderly patients after hip fracture: An observational study using the Japan Rehabilitation Database

These data suggest that the participation of board-certificated physiatrists is associated with good rehabilitation outcomes in patients with hip fracture at convalescent rehabilitation wards.

Others

1. Comparison of functional outcome between lacunar infarction (LI) and branch atheromatous disease (BAD) in lenticulostriate artery territory

These data demonstrated that BAD patients can obtain activities of daily living similar to LI patients. However, many BAD patients require canes and/or orthoses.

2. Validation of the "activity and participation" component of the International Classification of Functioning, Disability and Health (ICF) Core Sets for stroke patients in Japanese rehabilitation wards

The "d" component of these 2 ICF Core Sets reflected functional status and disability. They could be a valid measure in post-acute stroke patients in the rehabilitation setting.

Publications

Kakuda W, Abo M, Sasanuma J, Shimizu M, Okamoto T, Kimura C, Kakita K, Hara H. Combination protocol of low-frequency rTMS and intensive occupational therapy for post stroke upper limb hemiparesis: a 6-year experience of more than 1700 Japanese patients. *Transl Stroke Res.* 2016; **7:** 172-9.

Hara T, Abo M, Kakita K, Masuda T, Yamazaki R. Dose a combined intervention program of repetitive transcranial magnetic stimulation and intensive occupational therapy affect cognitive function in patients with post-stroke upper limb hemiparesis? *Neural Regen Res.* 2016; **11:** 1932-9.

Hara T, Abo M, Kakita K, Mori Y, Yoshida M, Sasaki N. The effect of selective trancranial magnetic stimulation with functional near-infrared spectroscopy and intensive speech therapy on individuals with post-stroke aphasia. *Eur Neurol.* 2017; **77:** 186-94.

Sasaki N, Abo M, Hara T, Yamada N, Niimi M, Kakuda W. High-frequency rTMS on leg motor area in the early phase of stroke. *Acta Neurol Belg.* 2017; **117:** 189-94. Epub 2016 Aug 9.

Kakuda W, Momosaki R, Yamada N, Abo M. High-freaquency rTMS for the treatment of chronic fatigue syndrome: a case series. *Intern Med.* 2016; **55:** 3515-9.

Momosaki R, Kakuda W, Yamada N, Abo M. Influence of repetitive peripheral magnetic stimulation on neural plasticity in the motor cortex related to swallowing. *Int J Rehabil Res.* 2016; **39:** 263-6.

Momosaki R, Kinoshita S, Kakuda W, Yamada N, Abo M. Noninvasive brain stimulation for dysphagia after acquired brain injury: A systematic review. *J Med Invest.* 2016; **63:** 153-8.

Yamada N, Kakuda W, Yamamoto K, Momosaki R, Abo M. Atomoxetine administration combined with intensive speech therapy for post-stroke aphasia: evaluation by a novel SPECT mrthod. *Int J Neurosci.* 2016; **126:** 829-38.

Nakayama Y, Iijima S, Kakuda W, Abo M. Effect of home-based training using a slant board with dorsiflexed ankles on walking function in post-stroke hemiparetic patients. *J Phys Ther Sci.* 2016; **28:** 2353-7.

Momosaki R, Yasunaga H, Matsui H, Horiguchi H, Fushimi K, Abo M. Predictive factors for oral intake after aspiration pneumonia in older adults. *Geriatr Gerontol Int.* 2016; **16:** 556-60.

Momosaki R, Yasunaga H, Matsui H, Fushimi K, Abo M. Proton pump inhibitors versus histamine-2 receptor antagonists and risk of pneumonia in patients with acute stroke. *J Stroke Cerebrovasc Dis.* 2016; **25:** 1035-40.

Momosaki R, Kakuda W, Yamada N, Abo M. Impact of board-certificated physiatrists on rehabilitation outcomes in elderly patients after hip feacture: An observational study using the Japan Rehabilitation Database. *Geriatr Gerontol Int.* 2016; **16:** 963-8.

Niimi M, Abo M, Miyano S, Sasaki N, Hara T, Yamada N. Comparison of functional outcome between lacunar infarction and branch atheromatous disease in lenticulostriate artery territory. *J Stroke Cerebrovasc Dis.* 2016; **25:** 2271-5.

Kinoshita S, Abo M, Miyamura K, Okamoto T, Kakuda W, Kimura I, Urabe H. Validation of the "Activity and participation" component of ICF Core Sets for stroke patients in Japanese rehabilitation wards. *J Rehabil Med.* 2016; **48:** 764-8.

Department of Emergency Medicine

Satoshi Takeda, *Professor*
Kei Ohtani, *Associate Professor*
Taro Nameki, *Assistant Professor*
Masahiko Uzura, *Professor*
Kenji Okuno, *Associate Professor*
Takeki Ogawa, *Emeritus*

General Summary

1. Education system for junior residents in Emergency Medicine
2. Establishing a database of severe traumatic brain injury in Japan
3. The etiology of syncope
4. Research on laboratory assessment of myocardial infarction in the emergency room
5. Managing the course of Immediate Cardiac Life Support (ICLS)
6. Managing the course of Japan Advanced Trauma Evaluation and Care
7. Providing logistical support to the Japan Boxing Commission
8. Basic research of traumatic brain injury
9. Basic and clinical researches of oxidative stress and emergency medicine
10. Advice to local authorities on plans for disaster medicine
11. Creation of DMAT (Disaster Medical Assistance Team) deployment system
12. Management of hospital emergency response drill including Code Blue (Stat Call) and Rapid Response System (RRS)
13. Managing the Jikei Airway Management course for Patient safety (JAMP)
14. Providing logical support for the Japan AED Foundation

Research Activities

1. Supervision and development of ultrasound devices in the diagnosis and treatment of cerebrovascular disorders
2. Director of Japan Neurotrauma Data Bank Committee
3. Prognostic value of heart fatty acid–binding protein for patients with chest symptoms in the emergency room
4. Research committee on higher cerebral function after traumatic brain injury
5. Research committee on impact biomechanics in automobile accidents (Society of Automotive Engineers of Japan, Inc.)
6. Published a revised edition of *Guidelines for the Treatment and Management of Severe Head Injury* (The Japan Society of Neurotraumatology)
7. Research group on cerebrospinal fluid in cases of traumatic intracranial hypotension
8. Management of the Japan Advanced Trauma Evaluation and Care Course
9. Basic research of traumatic brain injury and oxidative stress
10. Basic research of heat stroke and neuronal injury
11. Development of anti free radical therapy in patients with acute neuronal conditions
12. Development of educational system in Emergency Medicine including usage of simulation training

Publications

Mitsunaga T, Ohtani K, Dohi K, Kiriyama N, Ohtaki Y, Satoh K, Takeda S, Ogawa T. Beneficial Effects of Infused Acetated Ringer's Solution Containing Glucose and Ionized Magnesium on Patients with Acute Alcohol Intoxication. *Arch Emerg Med Crit Care.* 2016; **1:** 1012.

Wang Z, Komatsu T, Mitsumura H, Nakata N, Ogawa T, Iguchi Y, Yokoyama M. An uncovered risk factor of sonothrombolysis: Substantial fluctuation of ultrasound transmittance through the human skull. *Ultrasonics.* 2017; **77:** 168–75.

Wang Z, Sawaguchi Y, Hirose H, Ohara K, Sakamoto S, Mitsumura H, Ogawa T, Iguchi Y, Yokoyama M. An In Vitro Assay for Sonothrombolysis Based on the Spectrophotometric Measurement of Clot Thickness. *J Ultrasound Med.* 2017; **36:** 681–98.

Department of Laboratory Medicine

Tomokazu Matsuura, *Professor*
Ken Kaito, *Professor*
Hironari Sue, *Professor*
Kouji Nakada, *Associate Professor*
Yoshihiro Mezaki, *Assistant Professor*
Setuko Akizuki, *Assistant Professor*
Akihiro Ohnishi, *Professor*
Hiroshi Yoshida, *Professor*
Kenichi Sugimoto, *Professor*
Takahiro Masaki, *Assistant Professor*
Midori Kono, *Assistant Professor*

General Summary

The environment for research in the department was equipped. The main study was accomplished by Welfare of Japan and the Practical Application of New Drugs for Hepatitis B provided by the Japan Agency for Medical Research and Development (AMED). We accomplished studies to connect experimental medicine with clinical medicine

Research Activities

Clinical microbiology

Basic and clinical research on microbes was conducted. 1) We assessed antiviral effects of hit compounds from a high-throughput screen using a novel *in vitro* HBV cell culture system. 2) We developed a cell-based HCV infection system with a highly sensitive Gaussia luciferase reporter for monitoring of viral RNA replication. By using this cell-based HCV infection assay and a functional miRNA library screen, we identified a miRNA involved in HCV replication. 3) We investigated the prevalence of extended spectrum β-lactamase-producing Enterobacteriaceae in fecal samples collected from outpatients, in collaboration with the Department of Clinical Laboratory, Tokyo Metropolitan Bokutoh Hospital.

We investigated characterization for differentiation at the strains level of *Helicobacter cinaedi* isolated from blood culture using multilocus sequence typing (MLST) method. The most common strain type was ST10.

We did try to perform an epidemiologic study of MRSA using a whole cell MALDI TOF MS.

Clinical chemistry

We studied gastric emptying and fat digestive and absorptive function after various types of gastrectomy by ^{13}C-breath tests. Function-preserving gastrectomy (pylorus-preserving gastrectomy; PPG, proximal gastrectomy) attenuated rapid gastric emptying usually seen after conventional gastrectomy, which may in turn ameliorate postgastrectomy syndrome such as diarrhea and dumping.

We demonstrated the detail characteristic of diabetic dyslipidemia based on lipoprotein profile data measured by our developed HPLC lipoprotein assay (J Clin Med Res. 2016; 8: 424-6), and also reported the relevance of 6-factionated lipoprotein profile with Lp(a) to coronary heart disease risk score (J Atheroscler Thromb. 2016 Dec 26 [Epub ahead of

print]).
In a study on clinical laboratory specimens, we continued our research on nonspecific reactions and published papers on autoantibodies affecting immunoassays.

Clinical oncology
Clinico-pathological evaluation of huge splenomegaly:
Characteristics of huge splenomegaly were analyzed. Underlying disease was myelofibrosis (2), polycythemia (2), splenic lymphoma (1), chronic hemolysis (1), lymphoplasmacytic lymphoma (1). JAK2 mutation, lymphocyte infiltration, and destruction of blood were major cause of splenomegaly.

Clinical physiology
In the field of physiological examination, we examined accuracy of arrhythmia diagnosis of automatic analysis of electrocardiogram. In the arrhythmic region, we continued research related to catheter ablation of atrial fibrillation and reported it to English journals.

Clinical psychiatry
Taking Neojacksonism (Ey H) into consideration, we discussed the interpretation about the cases that presented the psychotic symptoms associated with epilepsy. Furthermore we reported changes in serum concentrations of AEDs (especially new-antiepileptic drugs) during pregnancy of epileptic patients. A study was performed to prevent the recurrence of depression in patients with epilepsy.

Basical Research
Hepatic stellate cells are the major site of retinoid storage and their activation is a key process in liver fibrogenesis. Vitamin A exerts most of its physiological activities through transcriptional regulation by retinoic acid receptors. We proposed that speckled cytosolic distribution of retinoic acid receptor proteins represents a new marker of hepatic stellate cell activation.

Publications

Horikiri T, Hara H, Saito N, Araya J, Takasaka N, Utsumi H, Yanagisawa H, Hashimoto M, Yoshii Y, Wakui H, Minagawa S, Ishikawa T, Shimizu K, Numata T, Arihiro S, Kaneko Y, Nakayama K, Matsuura T, Matsuura M[1], Fujiwara M[2], Okayasu I[3], Ito S[4], Kuwano K ([1]Teikyo Univ, [2]Japanese Red Cross Med Center, [3]Kitasato Univ Sch of Med, [4]The Univ of Tokyo). Increased levels of prostaglandin E-major urinary metabolite (PGE-MUM) in chronic fibrosing interstitial pneumonia. *Respir Med.* 2017; **122:** 43-50. Epub 2016 Now 24.

Altinel K[1], Hashimoto K[1], Wei Y[2], Neuveut C[2], Gupta I[1], Suzuki AM[1], Dos Santos A[3,4], Moreau P[2], Xia T[2], Kojima S[1], Kato S[1], Takikawa Y[5], Hidaka I[6], Shimizu M[7], Matsuura T, Tsubota A, Ikeda H[8], Nagoshi S[9], Suzuki H[1], Michel ML[2], Samuel D[3,4], Buendia MA[3,4], Faivre J[3,4], Carninci P[1] ([1]RIKEN, [2]Institut Pasteur, [3]Paul-Brousse Hosp, [4]Univ Paris Sud, [5]Iwate Med Univ, [6]Yamaguchi Univ Grad Sch of Med, [7]Gifu Univ Grad Sch of Med, [8]The Univ of Tokyo, [9]Saitama Med Univ). Single-nucleotide-resolution mapping of HBV promoters in infected human livers and hepatocellular carcinoma. *J Virol.* 2016; **90:** 10811-22.

Masubuchi N[1], Sugihara M[1], Sugita T, Amano K, Nakano M, Matsuura T ([1]Daiichi Sankyo Co., Ltd). Oxidative stress markers, secondary bile acids and sulfated bile acids classify the clini-

cal liver injury type : Promising diagnostic biomarkers for cholestasis. *Chem Biol Interact.* 2016; **255:** 83–91.

Hioki M, Matsuo S, Tokutake K, Yokoyama K, Narui R, Ito K, Tanigawa S, Tokuda M, Yamashita S, Anan I, Inada K, Sakuma T, Sugimoto KI, Yoshimura M, Yamane T. Filling defects of the left atrial appendage on multidetector computed tomography: their disappearance following catheter ablation of atrial fibrillation and the detection of LAA thrombi by MDCT. *Heart Vessels.* 2016; **31:** 2014–24.

Isozaki H[1], Matsumoto S[1], Murakami S[1], Takama T[1], Sho T[1], Ishihara K[1], Sakai K[1], Takeda M[2], Nakada K, Fujiwara T[3] ([1]Oomoto Hospital, [2]Watanabe Hospital, [3]Okayama Univ). Diminished Gastric Resection Preserves Better Quality of Life in Patients with Early Gastric Cancer. *Acta Med Okayama.* 2016; **70:** 119–30.

Konishi H, Nakada K, Kawamura M, Iwasaki T, Murakami K, Mitsumori N, Yanaga K. Impaired Gastrointestinal Function Affects Symptoms and Alimentary Status in Patients After Gastrectomy. *World J Surg.* 2016; **40:** 2713–18.

Nakada K, Takahashi M[1], Ikeda M[2], Kinami S[3], Yoshida M[4], Uenosono Y[5], Kawashima Y[6], Nakao S[7], Oshio A[8], Suzukamo Y[9], Terashima M[10], Kodera Y[11] ([1]Yokohama Municipal Citizen's Hospital, [2]Asama General Hospital, [3]Kanazawa Medical School, [4]International University of Health and Welfare Hospital, [5]Kagoshima Univ, [6]Saitama Cancer Center, [7]Tokyo Women's Medical Univ, [8]Waseda Univ, [9]Tohoku Univ, [10]Shizuoka Cancer Center, [11]Nagoya Univ). Factors affecting the QOL of patients after gastrectomy as assessed using the newly developed PGSAS-45 scale: a nationwide multi-institutional study. *World J Gastroenterol.* 2016; **22:** 8978–90.

Nitta S[1], Asahina Y[1], Matsuda M[2], Yamada N[2], Sugiyama R[3], Masaki T, Suzuki R[2], Kato N[3], Watanabe M[1], Wakita T[2], Kato T[2] ([1]Tokyo Medical and Dental Univ, [2]National Institute of Infectious Diseases, [3]Okayama Univ). Effects of resistance-associated NS5A mutations in hepatitis C virus on viral production and susceptibility to antiviral reagents. *Sci Rep.* 2016; **6:** 34652.

Sakaguchi M[1], Manabe N[2], Ueki N[3], Miwa J[4], Inaba T[5], Yoshida N[6], Sakurai K[7], Nakagawa M[8], Yamada H[9], Saito M[10], Nakada K, Katsuhiko Iwakiri K[11], Joh T[12], Haruma K[2] ([1]Moriguchi Keijinkai Hosp, [2]Kawasaki Medical Sch, [3]Tokyo Rosai Hosp, [4]Toshiba General Hosp, [5]Kagawa Prefectural Central Hosp, [6]Jpn Red Cross Kyoto Daiichi Hosp, [7]Hattori Clinic, [8]Hiroshima City Hosp, [9]Shinko Hosp, [10]Michiya Clinic, [11]Nippon Medical Sch, [12]Nagoya City Univ). Factors associated with complicated erosive esophagitis: A Japanese multicenter, prospective, cross-sectional study. *World J Gastroenterol.* 2017; **23:** 318–27.

Takahashi M[1], Terashima M[2], Kawahira H[3], Nagai E[4], Uenosono Y[5], Kinami S[6], Nagata Y[7], Yoshida M[8], Aoyagi K[9], Kodera Y[10], Nakada K ([1]Yokohama Municipal Citizen's Hosp, [2]Shizuoka Cancer Center, [3]Chiba Univ, [4]Kyushu Univ, [5]Kagoshima Univ, [6]Kanazawa Med Sch, [7]Nagasaki Univ, [8]Int Univ Health Welfare Hosp, [9]Kurume Univ, [10]Nagoya Univ). Quality of life after total vs distal gastrectomy with Roux-en-Y reconstruction: Use of the Postgastrectomy Syndrome Assessment Scale-45. *World J Gastroenterol.* 2017; **23:** 2068–76.

Narui R, Matsuo S, Isogai R, Tokutake K, Yokoyama K, Kato M, Ito K, Tanigawa SI, Yamashita S, Tokuda M, Inada K, Shibayama K, Miyanaga S, Sugimoto K, Yoshimura M, Yamane T. Impact of deep sedation on the electrophysiological behavior of pulmonary vein and non-PV firing during catheter ablation for atrial fibrillation. *J Interv Card Electrophysiol.* 2017; **49:** 51–7.

Reviews and Books

Yanai H, Hirowatari Y, Ito K, Kurosawa H, Tada N, Yoshida H. Understanding of Diabetic Dyslipidemia by Using the Anion-Exchange High Performance Liquid Chromatography Data. *J Clin Med Res.* 2016; **8:** 424–6.

Manita D, Yoshida H, Hirowatari Y. Cholesterol Levels of Six Fractionated Serum Lipoproteins and its Relevance to Coronary Heart Disease Risk Scores. *J Atheroscler Thromb.* 2017 Sep 1; **24:** 928–939. Epub 2016 Dec 26.

Kishimoto Y, Yoshida H, Kondo K. Potential Anti-Atherosclerotic Properties of Astaxanthin. *Mar Drugs.* 2016 Feb 5; **14:** pii: E35.

de Carvalho LS, Yoshida H. Monthly PCSK9 inhibitors: The CHOICE for prolonged duration of effect. *Atherosclerosis.* 2016; **254:** 300–2.

Department of Endoscopy

Kazuki Sumiyama, *Professor*
Keiichi Ikeda, *Associate Professor*
Kenichi Goda, *Assistant Professor*
Naoto Tamai, *Assistant Professor*
Hiroo Imazu, *Professor*
Hiroshi Arakawa, *Assistant Professor*
Hirobumi Toyoizumi, *Assistant Professor*

General Summary

Our main area of research is performing clinical studies of endoscopy for the diagnosis and treatment of gastrointestinal (GI), hepatobiliary, and pancreatic diseases. In addition, we perform basic research to develop novel instrumentation, methods of image processing and analysis, and optical apparatuses, such as autofluorescence imaging (AFI), narrow-band imaging (NBI), endocytoscopy, confocal laser endomicroscopy, and therapeutic endoscopy with a high degree of procedural freedom. Our published research outcomes and recent reports are summarized below.

Research Activities

Pharyngeal, esophageal, gastric, duodenal and colonic malignancies

1. Endoscopic diagnosis of neoplasia in the GI tract

Early detection and accurate diagnosis of premalignant and malignant lesions in the GI tract are essential to allow the most appropriate therapeutic strategy to be selected for each patient. To evaluate these clinical cases we use several novel optical technologies, along with conventional white light endoscopy. We have designed a series of prospective clinical studies to evaluate and validate these novel imaging technologies and their potential benefits.

a) Magnifying endoscopic observation with an NBI system

This new diagnostic system consists of a magnifying (× 90) endoscope and a NBI light source, which provides detailed morphological information about the capillaries on the mucosal surface of neoplastic lesions. We are investigating the clinical utility of NBI-magnifying endoscopy for identifying superficial neoplasms and developing algorithms that would allow determination of the histological type and tumor extent of GI tract neoplasia. On the basis of our findings with magnified NBI, we have developed a novel classification system for gastric cancer and demonstrated, in a prospective study, its advantages over the conventional diagnostic system. We also joined a multicenter study of NBI-magnifying endoscopy for detecting superficial carcinomas of the pharynx and esophagus. Accurate preoperative evaluation of the depth of invasion into the submucosal layer is essential for appropriate decision-making and for determining the optimal therapeutic strategy for patients with colonic lesions. To maximize our diagnostic accuracy, we use this magnifying endoscope with NBI and crystal-violet staining for this purpose. Results of these studies have been reported at several conferences and have been published in several English-language journals.

b) Endomicroscopy

Endocytoscopy is a novel optical imaging technique that allows the gastrointestinal mucosa to be visualized in vivo and in real time at a cellular level. We are now studying the characteristic endocytoscopy findings of superficial duodenal neoplasms, i.e., adenoma and mucosal adenocarcinoma. We also introduced confocal laser endomicroscopy, which provides subsurface imaging of the GI walls with image quality equivalent to that of bench confocal microscopy. We have joined an international multicenter study to assess the advantages of the confocal laser endomicroscopy over conventional endoscopy for differentiating gastric neoplasia from nonneoplastic mucosa. The results were reported at international meetings and published in an internationally renowned scientific journal.

c) AFI endoscopic system

The AFI endoscopic system has recently been developed to visualize autofluorescence emitted from the gastrointestinal wall. Theoretically, AFI can be used to detect premalignant lesions or early-stage malignancies that do not have a distinct appearance on conventional white-light endoscopy. Although AFI remains associated with a high false-positive rate, we established that AFI, in combination with conventional white-light imaging and NBI, could improve specificity.

2. Endoscopic treatment of esophageal, gastric, and colonic malignancies

Recent advances in endoscopic diagnostic techniques and instrumentation have led to the expansion of indications for endoscopic therapy in GI tract carcinomas. We now perform endoscopic submucosal dissection (ESD) for superficial neoplasms of the esophagus, stomach, and colon. En bloc resection with ESD is considered necessary to further develop endoscopic treatment. Successful development of a series of endoscopic knives traction devices and submucosal injection fluids reduced the technical difficulty of ESD and the risk of complications.

3. Diagnosis and treatment of oropharyngeal and hypopharyngeal malignancies

Detecting cancer at an early stage is important. We have found that, in combination with the NBI system, magnifying endoscopy has allowed hard-to-find cancers to be detected during their early stages, without the need for Lugol chromoendoscopy. In collaboration with department of otorhinolaryngology, we also introduced endoscopic removal of early-stage cancers in this area and are evaluating clinical outcomes.

Enteroscopy

1. Diagnostic techniques

Capsule endoscopy is a breakthrough modality that can be used to detect lesions in parts of the small intestine that are inaccessible with an ordinary endoscope system. However, because capsule endoscopy is purely diagnostic, we have introduced single-balloon enteroscopy, which allows biopsy and hemostasis to be performed for hemorrhagic lesions of the small intestine.

Pancreatobiliary endoscopy

1. Diagnosis of biliary and pancreatic diseases

The establishment of a standardized, systematic diagnostic algorithm for biliary and pancreatic diseases are extremely important. We are comparing the diagnostic accuracy of endoscopic ultrasound (EUS)-guided fine needle aspiration biopsy, multidetector-row

computed tomography, magnetic resonance cholangiopancreatography, and endoscopic retrograde cholangiopancreatography in hepatopancreatic diseases. We are developing new diagnostic markers for pancreatic carcinoma and a system for their measurement. We will be applying these markers to the differential and prognostic diagnosis of pancreatic carcinoma with specimens obtained with EUS-guided fine needle aspiration biopsy.

2. Treatment using endoscopic techniques in pancreatobiliary diseases

The technique of EUS-guided celiac plexus block has been performed to control persistent pain due to chronic pancreatitis, even in benign disease. We have performed EUS-guided celiac plexus neurolysis using a small amount of injected ethanol and are now evaluating the feasibility of this approach. We have also started animal experiments to develop new interventional technologies with EUS and microbubbles to locally control pancreatic cancer.

Publications

Goda K, Dobashi A, Yoshimura N, Aihara H, Kato M, Sumiyama K, Toyoizumi H, Kato T, Saijo H, Ikegami M, Tajiri H. Dual-focus versus conventional magnification endoscopy for the diagnosis of superficial squamous neoplasms in the pharynx and esophagus: a randomized trial. *Endoscopy.* 2016 Apr; **48:** 321-9.

Tamai N, Inomata H, Ide D, Dobashi A, Saito S, Sumiyama K. Effectiveness of color intensity analysis using updated auto-fluorescence imaging systems for serrated lesions. *Dig Endosc.* 2016 Apr; **28:** 49-52.

Matsuda T, Oka S, Ikematsu H, Matsushita HO, Mori Y, Takeuchi Y, Tamai N, Kawamura T, Chino A, Keum B, Khomvilai S, Uraoka T. Endoscopic diagnosis of colorectal serrated lesions: Current status and future perspectives based on the results of a questionnaire survey. *Dig Endosc.* 2016 Apr; **28:** 35-42.

Hamada K, Takeuchi Y, Tamai N. A case of sessile serrated adenoma/polyp observed with autofluorescence imaging. *Dig Endosc.* 2016 Apr; **28:** 61.

Kobayashi M, Neumann H, Hino S, Vieth M, Abe S, Nakai Y, Nakajima K, Kiesslich R, Hirooka S, Sumiyama K. Influence of reviewers' clinical backgrounds on interpretation of confocal laser endomicroscopy findings. *Endoscopy.* 2016 Jun; **48:** 521-9.

Dobashi A, Sumiyama K, Gostout CJ. Simplified prophylaxis of mucosal resection site defects. *Gastrointest Endosc.* 2016 Jun; **83:** 1265-6.

Sano Y, Tanaka S, Kudo SE, Saito S, Matsuda T, Wada Y, Fujii T, Ikematsu H, Uraoka T, Kobayashi N, Nakamura H, Hotta K, Horimatsu T, Sakamoto N, Fu KI, Tsuruta O, Kawano H, Kashida H, Takeuchi Y, Machida H, Kusaka T, Yoshida N, Hirata I, Terai T, Yamano HO, Kaneko K, Nakajima T, Sakamoto T, Yamaguchi Y, Tamai N, Nakano N, Hayashi N, Oka S, Iwatate M, Ishikawa H, Murakami Y, Yoshida S, Saito Y. NBI magnifying endoscopic classification of colorectal tumors proposed by the Japan NBI Expert Team (JNET). *Dig Endosc.* 2016 Jul; **28:** 526-33.

Hara H, Takahashi T, Nakatsuka R, Higashi S, Naka T, Sumiyama K, Miyazaki Y, Makino T, Kurokawa Y, Yamasaki M, Takiguchi S, Mori M, Doki Y, Nakajima K. A novel approach of optical biopsy using probe-based confocal laser endomicroscopy for peritoneal metastasis. *Surg Endosc.* 2016 Aug; **30:** 3437-46.

Dobashi A, Goda K, Yoshimura N, Ohya TR, Kato M, Sumiyama K, Matsushima M, Hirooka S, Ikegami M, Tajiri H. Simplified criteria for diagnosing superficial esophageal squamous neoplasms using Narrow Band Imaging magnifying endoscopy. *World J Gastroenterol.* 2016 Nov 7; **22:** 9196-204.

Niikura R, Nagata N, Doyama H, Ota R, Ishii N, Mabe K, Nishida T, Hikichi T, Sumiyama K, Nishikawa J, Uraoka T, Kiyotoki S, Fujishiro M, Koike K. Current state of practice for colonic diverticular bleeding in 37 hospitals in Japan: A multicenter questionnaire study. *World J Gastrointest Endosc.* 2016 Dec 16; **8:** 785-94.

Kawamura T, Oda Y, Kobayashi K, Matsuda K, Kida M, Tanaka K, Kawahara Y, Yokota I, Koizumi W, Yasuda K, Tajiri H. Colonoscopy Withdrawal Time and Adenoma Detection Rate: A Japanese Multicenter Analysis. *Journal of GHR.* 2017.02; **6:** 2273-8.

Sumiyama K. Past and current trends in endoscopic diagnosis for early stage gastric cancer in Japan. *Gastric Cancer.* 2017 Mar; **20:** 20-7.

Sumiyama K, Kobayashi M. Enteric nervous system visualization with cresyl violet-assisted confocal laser endomicroscopy — Progress Overview FY2016 — . The 3rd international Symposium on the project "Multidisciplinary Computational Anatomy". 2017 Mar; 205-7.

Department of Infection Control

Seiji Hori, *Professor*
Tetsuya Horino, *Associate Professor*
Yasushi Nakazawa, *Assistant Professor*
Masaki Yoshida, *Associate Professor*
Hiroshi Takeda, *Assistant Professor*
Koji Yoshikawa, *Assistant Professor*

General Summary

We performed both basic and clinical research in the following areas: bacterial infection and chemotherapy, opportunistic infection in patients with human immunodeficiency virus/acquired immunodeficiency syndrome, parasitic/vector borne diseases, and outbreak and infection control.

Research Activities

Clinical studies on patients with bacteremia due to methicillin-resistant Staphylococcus aureus

We investigated the clinical features and treatment of 32 patients with bacteremia due to methicillin-resistant *S. aureus* (MRSA). Catheter-related bloodstream infection accounted for 59.4% of source of infection. Since 2013, the number of MRSA bacteremia cases has decreased and the infection control team (ICT) intervention rate for MRSA bacteremia has increased. Therapeutic Drug Monitoring (TDM) was performed in all cases who vancomycin and teicoplanin was used for treatment. Some cases were changed to daptomycin or linezolid after prolonged use of initial treatment. ICT needs to actively intervene even after the start of treatment in addition to selecting anti-MRSA agents.

Interaction between integrase strand transfer inhibitors and calcium-free phosphate-binding agents in HIV patients on hemodialysis

The aim of this study was to determine the interaction between integrase strand transfer inhibitors (INSTIs) and calcium-free phosphate-binding agents. Blood samples were collected from two HIV patients with chronic kidney disease on hemodialysis. Patient 1 was administered dolutegravir and bixalomer, and Patient 2 was raltegravir and lanthanum carbonate. There was no significant reduction of the blood concentrations of dolutegravir or raltegravir. However, the time to maximum concentration of raltegravir was delayed in Patient 2. Therefore, further investigation will be needed to clarify the reason for this delay and the interaction between concomitantly administered INSTIs and calcium-free phosphate-binding agents.

Revisiting a method for diagnosing toxoplasmosis: Development of the Toxoplasma Killing Observation test

Toxoplasma gondii, the most successful protozoan infects approximately 1/3 of people worldwide. In most cases, Toxoplasmosis is self-limited disease with mild symptoms, even asymptomatic, except immunocompromised patient and pregnant women. Immuno-

compromised patients, such as AIDS, post organ transplant patients and steroid users are at the risk of Toxoplasma encephalitis, pneumonitis and retinitis. Documents show that most of these cases were caused by relapses and flares from the bradizoite; a slowly duplicating form of Toxoplasma, which dormant in host tissues. On the other hand, primary infection during pregnancy is a risk of congenital Toxoplasmosis, which causes intrauterine growth retardation, hydrocephaly, mental growth retardation, retinitis, and even fetal death.

Many types of serodiagnostic methods are widely used for the detection of Toxoplasmosis over the world. But in Japanese clinical site, Toxoplasma immunoglobulin G and M is the only serodiagnosis method that makes diagnosis complicated in some cases. In relapsed patients IgM is usually negative, and positive IgG could not discriminate the present infection from the past infection. Diagnosis of congenital toxoplasmosis with IgG and IgM are another issue. Some documents report that IgM remains over the threshold for more than two years, and the positive predictive value of IgM was only 45.98%. This kinetics of IgM has a risk of leading misdiagnosis.

Sabin and Feldman reported dye test in 1948. Dye test evaluates the aggregate ability of tachyzoite-cidal immunoglobulin titer with the serum of the subject. The classic serodiagnosis still owns high sensitivity and specificity as a referential diagnosis. Issue of the dye test is its complicated evaluation method that evaluator must count stained tachyzoites under visual recognition. We tackled this issue with a green fluorescent protein expressed tachyzoite, which is the alternative marker for evaluating deactivation of the tachyzoite. The new improved dye test, Toxoplasma killing observation (TOKIO) test has advantage of its objectivity and retention for evaluation, and equivalent outcome as classical dye test.

Seroepidemiology and risk assessment of Toxoplasma gondii infection in HIV/AIDS patients

In HIV-infected patients, AIDS develops with decreased CD4 positive lymphocytes. Toxoplasma encephalitis is one of the AIDS indicator diseases that its risk increases when CD4 positive lymphocyte becomes 100/μl or less. Majority of cases are caused by reactivation of bradizoites in brain, which forms latent infection. However, there is no adequate assessment of toxoplasma seroprevalences and its risk factors among Japanese HIV-infected patients. We collected serums from 400 HIV-infected patients who visited our hospital outpatient clinic and conducted serological evaluation of *T.gondii* specific-antibody levels. As a result, 33 cases (8.3%) of patients were T.gondii IgG antibody positive, and confirmed positive by Sabin-Feldman Dye test. The obtained prevalence of seropositivity was equivalent to the previous survey that was conducted in pregnant women in Japan; there was no correlation with HIV infection. Also, a strong correlation with a history of cat rearing was found, rather than having a habit of consuming rare meat from the questionnaire survey to the participants.

Disinfection effect of Chlorhexidine gluconate and Olanexidine gluconate on clinically isolated S.aures *strains*

We investigated the disinfecting effect of chlorhexidine gluconate (CHG) and olanexidine

gluconate (OLN) on clinically isolated Staphylococcus aureus strains. OLN had higher disinfecting effect on clinically isolated *S. aureus* strain than CHG.

Publications

Nakaharai K, Sakamoto Y[1], Yaita K[2], Yoshimura Y[1], Igarashi S[3], Tachikawa N[1] ([1]Yokohama Municipal Citizen's Hospital, [2]Kurume University, [3]Yokohama Brain and Spine Center). Drug-induced liver injury associated with high-dose ceftriaxone: a retrospective cohort study adjusted for the propensity score. *Eur J Clin Pharmacol.* 2016; **72:** 1003-11.

Matsumoto K[1], Kurihara Y[1], Kuroda Y[1], Hori S, Kizu J[1] ([1]Keio University). Pharmacokinetics and brain penetration of carbapenems in mice. *J Infect Chemother.* 2016; **22:** 346-9.

Department of Dentistry

Katsuhiko Hayashi, *Professor*
Shigeru Suzuki, *Associate Professor*
Akihiro Ikai, *Professor*

General Summary

1. Role of dentistry in sleep apnea
2. The role of nerve growth factor and its precursor forms in oral wound healing
3. Clinical investigation of patients with medication-related osteonecrosis of the jaw in our department

Research Activities

Role of dentistry in sleep apnea

1. Oral appliance

Oral appliance (OA) which was one of the conservative treatment of OSA became the insurance adaptation from 2004. In a Japanese insurance adaptation standard, as for OA production, AHI $<$ 20 is recommended now. It is a premise that there are being diagnosed as being adaptation of OA in a medical department, the introduction from a doctor. However, OA serves as the supporting role of CPAP when the patient whom CPAP cannot use it. OSA is the breathing disorder that subsidence of the base of the tongue and stenosis of the cavity of pharynx caused by the soft palate produces during face up position sleep. OA plans an open size of the cavity of pharynx by raising a tongue forward and improves breathing disorder. In addition, it is confirmed that the daytime sleepiness lightens by OA treatment.

2. Sleep surgery

CPAP and OA are symptomatic treatments together, and an effect is maintained by using it continuously. In late years the sleep surgery attracts attention as basic treatment of OSA. Stanford University proposes Two Phase Surgery Protocol in 2007.

At first, in this protocol, the operation for soft tissues such as a nasal cavity, soft palate, the base of tongue is carried out as (phase1) for the first stage. When an effect of phase1 is insufficient, the operation for hard tissue particularly the jawbone is carried out as (Phase2) for the second stage. Dentist deals with Phase2. A method of surgery includes Genioglossal Advancement (GA) and Maxillo-Mandibuler Advancement (MMA).

3. Infant pediatric orthodontic

The cause of infant OSA is adenoids, enlargement of a lymphoid organization peculiar to an infant represented by hyperplasia of palatine tonsil and nose disease, hypogrowth of upper and lower jaws. In late years the effectiveness of the maxillary rapid expansion (RMA) for the infant OSA patient that the cause does not have adenoids increase and swollen tonsilsis reported in the United States. RMA magnifies upper jaw in a correction device rapidly and is an antidote to fix until an extended part ossifies.

Mechanism to give to the improvement of the upper respiratory tract ventilation disorder

of RMA in a recent study becomes clear. At present, RMA is a cure indicated for the infant whom a diagnosis of OSA established. However, when it is very likely to be the future OSA onset, in acknowledgment of a clear maxilla, I perform intervention of the teeth-straightening and acquire an appropriate chin face form for the infant period, and the way of thinking to prevent the OSA onset in the adulthood is suggested.

The Role of Nerve Growth Factor (NGF) and Its Precursor Forms in Oral Wound Healing

Nerve growth factor (NGF) and its different precursor forms are secreted into human saliva by salivary glands and are also produced by an array of cells in the tissues of the oral cavity. The major forms of NGF in human saliva are forms of pro-nerve growth factor (pro-NGF) and not mature NGF. The NGF receptors tropomyosin-related kinase A (TrkA) and p75 neurotrophin receptor ($p75^{NTR}$) are widely expressed on cells in the soft tissues of the human oral cavity, including keratinocytes, endothelial cells, fibroblasts and leukocytes, and in ductal and acinar cells of all types of salivary glands. In vitro models show that NGF can contribute at most stages in the oral wound healing process: restitution, cell survival, apoptosis, cellular proliferation, inflammation, angiogenesis and tissue remodeling. NGF may therefore take part in the effective wound healing in the oral cavity that occurs with little scarring. As pro-NGF forms appear to be the major form of NGF in human saliva, efforts should be made to study its function, specifically in the process of wound healing. In addition, animal and clinical studies should be initiated to examine if topical application of pro-NGF or NGF can be a therapy for chronic oral ulcerations and wounds.

Clinical investigation of patients with medication- related osteonecrosis of the jaw in our department

Bisphosphonate preparations are used for the prevention and treatment of bone related events such as bone metastasis of solid cancer, paraneoplastic hypercalcemia, multiple myeloma, and bone metabolism diseases such as osteoporosis. However, the bisphosphonate-related osteonecrosis of the jaw (BRONJ), which is an adverse event, is refractory to treatment for scratching of necrotic tissue and antibiotics therapy etc. Recently, osteonecrosis of jaw associated with novel therapeutic agents such as anti-RANKL antibody preparation (denosumab) and angiogenesis inhibitor (bevacizumab, sunitinib), which have different mechanisms of action from BP preparations, have also been reported, that is commonly referred to as Medication-Related osteonecrosis of the jaw (MRONJ).

The subjects were 24 patients diagnosed as MRONJ after seeing Department of Dentistry, Jikei University School of Medicine from January 2014 to January 2016. Among the 24 cases, the primary disease was breast cancer and prostate cancer with each 14 patients. In the administration route, 18 with injections and 6 with oral drugs were administered, among them 7 cases of new therapeutic agent only administration. By site, there were 20 cases of mandible and 4 cases of maxilla, almost all cases of chemotherapy, steroid therapy, or diabetes among risk factors were recognized. In stage classification, 4 cases of I, 14 cases of II, 6 cases of III. There were 14 cases of spontaneous onset and 10 cases after surgical treatment as a trigger for onset. It was suggested that with patients receiving bone

resorption suppressive drugs or angiogenesis inhibitors, surgical invasion to the jawbone or bacterial infection may deeply involve in the onset of MRONJ.

Publications

Schenck K*[1]*, Schreurs O*[1]*, Hayashi K, Helgeland K*[1] *(*[1]Univ of Oslo).*** The Role of Nerve Growth Factor (NGF) and Its Precursor Forms in Oral Wound Healing. *Int J Mol Sci.* 2017; **18:** 1-12.

Department of Transfusion Medicine

Tetsunori Tasaki, *Professor* Yoko Kato, *Associate Professor*

General Summary

1. The incidence of transfusion-associated adverse events in clinical departments was reported by Dr. Fujii (Yamaguchi University). The data were obtained from 17 university hospitals, including our own facility. The report showed that 4.79% of pediatric inpatients who were transfused with allogeneic blood suffered adverse events. The next most frequent (3.84%) was in hematology wards. He speculated that the higher rates in the two wards were associated with the amount of platelets used.
2. Various adverse events, including nausea and fever, occur in patients who undergo hematopoietic stem cell transplantation (HSCT). It is speculated that these events are probably due to the simultaneous transfusion of anticoagulant and cryoprotectant (dimethyl sulfoxide). However, as the details of events or causes are obscure, standard measures for preventing events associated with HSCT have not been proposed. In 2015, Dr. Ohto (Fukushima Medical University) formed a study group consisting of 20 investigators who began gathering detailed information on HSCT. As of August 31, 2016, data from 1,132 HSCT patients were collected, including 50 patients from our hospital. The interim results indicate that adverse events overall occurred most frequently in patients undergoing allogeneic bone marrow transplantation (BMT). In peripheral blood stem cell transplantation (PBSCT), the most frequent events observed were a change in blood pressure, nausea or a drop of blood oxygen saturation (SpO_2). Information from detailed analysis of these data will suggest ways in which we can improve the safety of HSCT.
3. At the annual meeting of the Japan Society of Transfusion Medicine and Cell Therapy in 2016, we stressed the importance of participation of medical technologists in preoperative conferences. Predicting problems or outlining treatment principles for a patient beforehand is important for the proper preparation of blood products, a change that could contribute to the safety of the surgery.

Research Activities

1. A Health and Labor Science Research Grant supported a 3-year study (2013-2015) that established a diagnostic algorithm to assist in distinguishing between transfusion-associated acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO). Unfortunately, this guideline has not gained worldwide acceptance. To enhance both the utility of the guideline and the differential diagnosis of TRALI and TACO, we have started to collect data from patients with dyspnea associated with blood transfusion.
2. Apheresis techniques using a cell separator are well established and commonly used for the collection of mononuclear cells (MNCs) for the treatment of patients with hematological disease. Recently, the technique has been used to collect a patient's MNCs for dendritic cell immunotherapy. In our hospital, this method was introduced for the treatment

of glioblastoma. Based on our experience with 31 apheresis patients, we have sometimes noted adverse events such as numbness due to hypocalcemia during the procedure. Difficulties in communicating with a patient with a central nervous system disorder is likely a contributing cause. To perform apheresis safely, we are now developing an improved protocol for these patients.

3. A new multicenter collaborative study is now under consideration for the purpose of studying children's alloimmunity against red blood cell antigens. In Japan, little data are available on the production of alloantibodies in children. Revealing the characteristics of alloantibodies (such as antigen affinity) and their clinical significance will improve the safety of blood transfusion in children.

4. We are planning to introduce cryoprecipitates made from allogeneic fresh frozen plasma, or autologous fibrin glue for the treatment of surgical bleeding. Before that, matters of concern such as significance and the cost-effectiveness were discussed.

Publications

Akasaki Y, Kikuchi T, Homma S, Koido S, Ohkusa T, Tasaki T, Hayashi K, Komita H, Watanabe N, Suzuki Y, Yamamoto Y, Mori R, Arai T, Tanaka T, Joki T, Yanagisawa T, Murayama Y. Phase I/II trial of combination of temozolomide chemotherapy and immunotherapy with fusions of dendritic and glioma cells in patients with glioblastoma. *Cancer Immunol Immunother.* 2016; **65:** 1499–509.

Ohara Y[1], Ohto H[1], Tasaki T, Sano H[1], Mochizuki K[1], Akaihata M[1], Kobayashi S[1], Waragai T[1], Ito M[1], Hosoya M[1], Nollet KE[1], Ikeda K[1], Ogawa C[2], Kanno T[3], Shikama Y[1], Kikuta A[1] ([1]Fukushima Med Univ, [2]National Cancer Center, [3]Fukushima Red Cross Blood Center). Comprehensive technical and patient-care optimization in the management of pediatric apheresis for peripheral blood stem cell harvesting. *Transfus Apher Sci.* 2016; **55:** 338–43.

Department of Molecular Physiology
Division of Physical Fitness

Shigeru Takemori, *Professor and Director* Hideki Yamauchi, *Assistant Professor*

General Summary

Research activities in our division have been focused on the plasticity of skeletal muscle and preventive medicine against metabolic syndrome in terms of exercise physiology.

Research Activities

Chronic exercise with diet restriction prevents diabetes via inactivation of FoxO signal in skeletal muscle

WBN/Kob-Fatty (WKF) rats lack leptin receptor, and develop chronic pancreatitis and diabetes with obesity. We recently reported that diet restriction improves their hyperlipidemia, insulin resistance, and pancreatic dysfunction more effectively when combined with chronic exercise. We now investigated metabolic profiles and intracellular signals in their skeletal muscle to clarify synergistic effects of chronic exercise on diet restriction. Male WKF rats (age, 6 weeks) were divided into fatty-obese (fatty-control), fatty-diet restriction (fatty-DR), and fatty-diet restriction plus exercise (DR+Ex) groups. WBN/Kob rats were used as lean-control. Food intake of fatty-DR and fatty-(DR+Ex) groups was restricted to 69% and 70% of the fatty-obese group, respectively. The exercise of the fatty-(DR+Ex) group was voluntary wheel running. After 6 weeks of intervention, it was found that chronic exercise increased the expressions of proteins associated with glucose uptake and phospholylation, mitochondria biomarkers, and autophagy-related proteins in skeletal muscle. In addition, chronic exercise inhibited FoxO3 signal rather than FoxO1 signal, and accelerated PGC-1a protein expression. We concluded that the chronic exercise at DR condition improved metabolic functions of skeletal muscle and prevented diabetes via inactivation of the PGC-FoxO3a signaling pathway.

Repetitive low-intensity eccentric contraction has little deteriorating effect on sarcomere structure at a molecular level

Using x-ray diffraction method we have reported that eccentric contraction (ECC) of moderate intensity (elicited by 75 Hz stimulation) induces evident deterioration in sarcomere structure evidenced as marked decrease in the intensity of myosin layer-lines. In this study, we evaluated the effects of low intensity ECC. Plantar muscles under blood flow supply of 8-week F344 anesthetized male rats were electrically stimulated through nerve. Consecutive contractions of 300 msec duration were elicited 10 times at 3 s intervals at one of the following conditions; 100 Hz isometric (ISO), 50 Hz ECC, and 75 Hz ECC. To evaluate the effect of overall load, a group of muscles underwent 30 consecutive 50 Hz ECC (50 Hz ECC×30). The overall contraction load evaluated as force-time integral was 50 Hz ECC $<$ ISO $=$ 75 Hz ECC $<$ 50 Hz ECC×30. Force developing capac-

ity evaluated 1 h after the consecutive test contractions was ISO = 50 Hz ECC < 50 Hz ECC×30 < 75 Hz ECC. Although scarcely observable after 75 Hz ECC, myosin layer-lines after ISO, 50 Hz ECC, and 50 Hz ECC×30 were comparable with the control. Sarcomere deterioration represented by myosin layer-lines are sensitive to instantaneous force development, but not to overall contraction load. Therefore, in rehabilitation care, repetitive low-intensity exercise may be adoptable without inducing serious sarcomere deterioration.

Effects of polyamines on skeletal muscle

Polyamines are poly-cationic molecules which are indispensable for proliferation of the eukaryotic cells. The proposed roles of polyamines are the modulation of ion channels, nucleic acid packaging, signal transduction, cell proliferation and differentiation, as well as regulation of gene expression. In skeletal muscle, regulation of polyamine levels may be associated with muscle hypertrophy and atrophy, yet the underlying mechanisms are not established. Thus, we studied how polyamines affect the proliferation and differentiation of murine myoblast progenitor C2C12 cell line. Upon polyamine treatment of C2C12 cells during induction of myogenic differentiation, the number of myotubes significantly increased. Morphologically, polyamine-treated C2C12 cells exhibited elongated cell body with larger number of nuclei per cell. On the other hand, the polyamine did not have influence on myoblast proliferation. Furthermore, C57BL6 mice that have underwent transection of left sciatic nerve exhibited enhanced compensatory hypertrophy of the right hindlimb muscled by polyamine administration. These results demonstrate that polyamines may play an important role in myogenic differentiation rather than myoblasts proliferation.

Effect of polyamine on calcium dynamics and electrophysiological property of cardiac cells

Polyamines may be involved also in exercise induced cardiac hypertrophy as previous workers have reported. On the other hand, polyamines are reported to modulate biological functions of ionic channels so as to modify physiological excitability of cardiac cells. Therefore, increased polyamine concentration within cardiac cells may cause arrhythmia in hypertrophic hearts of athletes. To address this issue, intracellular calcium dynamics and electrophysiological activity of cardiac cells were monitored. In vitro calcium dynamics and electrophysiological activity of isolated cardiac cells were monitored by fluorescent dyes. Excitability of cardiac cells in vivo was evaluated by electrocardiograph of anesthetized 4 rats. Polyamines increased the duration of a spontaneous discharge of cardiac cells both in vitro and in vivo. Polyamine increased intracellular basal calcium concentration in isolated ordinary cardiac cells without corresponding membrane potential change. Amplitude of T-wave of electrocardiograph was increased by the addition of polyamine. Increased intracellular polyamine concentration in cardiac cells may affect hypertrophic hearts of athletes to modify electrophysiological activities.

Department of Cell Physiology
Division of Aerospace Medicine

Susumu Minamisawa, *Professor*

General Summary

Our main research interests are gravitational physiology and aerospace medicine.

Research Activities

Gravitational physiology and aerospace medicine

1. Elucidation of the re-adaptation of attitude control after the return from long-term space flight

Astronauts returning from a long stay in space will be observed to learn more about the adaptive processes in the somatosensory system and the lower limb skeletal muscles and to acquire data that could contribute to astronaut rehabilitation after returning from space. We are collaborating with the Japan Aerospace Exploration Agency (JAXA) to perform this research. In this experiment, astronauts staying for a long time in space will be studied to measure the following items before and after their stay in orbit:

(1) Comparison of muscle activation patterns in lower limb antagonistic muscles
(2) Blood flow measurement in the lower limb skeletal muscles
(3) Body sway balance measurement

We collected and are analyzing data from 5 astronauts. We have obtained a preliminary result that the combination of skeletal muscles that are actived while the body sway balance is maintained did not recover to a nomal combination, even months after the astronauts returned to Earth. On the other hand, the astronauts' gait motion recovered and they could walk normally immediately after they returned to Earth.

2. Biomedical analyses of human hair exposed to long-term space flight

As a sample for experimental analysis, human hair has many advantages. Hair matrix cells actively divide in a hair follicle and sensitively reflect physical conditions. The hair shaft has an advantage to record the metabolic conditions of the subject's environment. The environment of space differs from that of the Earth in many factors, such as microgravity, space radiation, and mental stresses. These factors often induce physiological changes in our body. Hair samples will give us useful physiological information to examine the effect of space flight. In space experiments, we believe that hair is a suitable biological specimen because no special hardware or handling is required. We have recently published a paper in *PLOS ONE* reporting the results of this experiment. In this paper, we demonstrate that in some astronauts, genes related to hair growth are upregulated during flight, suggesting that space flight inhibits cell proliferation in hair follicles. Regarding the results of hair shaft, we are preparing to submit the article for publishing to the international journal.

3. Effects of heat stress on skeletal muscle properties

Space flight causes the loss of muscle mass, particularly in antigravity muscles. Astronauts exercise for 2 hours almost every day on the International Space Station to prevent the negative adaptation of skeletal muscles. However, the effect is limited. Skeletal muscles are exposed to various stressors during and after exercise. These stressors activate intracellular signaling and strengthen skeletal muscles. We hypothesized that stressors might be insufficient during space flight, even if astronauts exercise well and if external stimuli, which induce activation of intracellular signaling in muscles, could be useful as another countermeasure for astronauts. We are now focusing on heat stress and studying its effect to maintain and increase skeletal muscle properties.

Publications

Indo HP*[1]*, Majima HJ*[1]*, Terada M, Suenaga S*[1]*, Tomita K*[1]*, Yamada S*[2]*, Higashibata A*[1]*, Ishioka N*[1]*, Kanekura T*[1]*, Nonaka I*[3]*, Hawkins CL*[4]*, Davies MJ*[5]*, Clair DK*[6]*, Mukai C*[2] *(*[1]*Kagoshima Univ,* [2]*JAXA,* [3]*NCNP,* [4]*Sydney Univ,* [5]*Copenhagen Univ,* [6]*Kntucky Univ). Changes in mitochondrial homeostasis and redox status in astronauts following long stays in space. *Scientific Reports.* 2016; **6:** 39015.

Department of Pathology
Division of Neuropathology

Masahiro Ikegami, *Professor and Director* Takahiro Fukuda, *Assistant Professor*

General Summary

Our research projects have concerned neurodegenerative disorders caused by the intracellular accumulation of abnormal proteins. We are also studying mouse models of neurodegenerative disorders and autopsy cases by means of standard morphologic analysis and molecular biological analysis.

Research Activities

Logopenic primary progressive aphasia with pathologies of Alzheimer's disease and diffuse Lewy body disease

Logopenic primary progressive aphasia (lvPPA), characterized by anomia, difficulty repeating complex sentences, and phonological errors, is a group of clinically, genetically and pathologically heterogeneous disorders. The majority of lvPPA patients have underlying Alzheimer's disease with asymmetric atrophy of the language-dominant hemisphere, neurofibrillary tangles, neuritic plaques, and neuronal loss focused on the inferior frontal gyrus, motor cortex, supramarginal gyrus and superior temporal gyrus. It remains unknown whether diffuse Lewy body disease causes lvPPA or not. We report a case of Alzheimer's disease with lvPPA with asymmetric left-sided atrophy in the left inferior frontal gyrus, and temporal lobe; diffuse Lewy body disease; mild argyrophilic grain disease; cerebral amyloid angiopathy; and traumatic brain injury. Pathologies of Alzheimer's disease should primarily cause lvPPA. However, diffuse Lewy body disease with numerous Lewy bodies and Lewy neurites might evolve into disproportionately mild lvPPA and transient motor parkinsonism given the patient's pathologies of diffuse Lewy body disease.

Aggregation and phosphorylation of α-synuclein with proteinase K-resistance in focal α-synucleinopathy predominantly localized to the cardiac sympathetic nervous system

Aggregates of α-synuclein, a major component of Lewy bodies (LBs) and Lewy neurites (LNs), are distributed throughout the nervous system, including the central nervous system (CNS), sympathetic ganglia, enteric nervous system (ENS), cardiac and pelvic plexuses, submandibular gland, adrenal medulla and skin, in incidental Lewy body disease (ILBD), Parkinson's disease (PD), dementia with Lewy bodies (DLB), and pure autonomic failure (PAF). Here we report focal α-synucleinopathy predominantly localized to the cardiac sympathetic nervous system (SNS). Aggregation and phosphorylation of α-synuclein with proteinase K (PK)-resistance developed predominantly in the cytoplasm and proximal axon of the postganglionic sympathetic neuron (PGSN).

A 67-year-old man without parkinsonism or autonomic symptoms died of well-differen-

tiated squamous carcinoma of the tongue. Post-mortem brain investigation revealed neither neuronal loss nor gliosis of the substantia nigra (SN), locus coeruleus (LC), intermediate reticular zone (IRZ), nor dorsal motor nucleus of the vagus nerve (DMV). No LBs were observed in the central nervous system (CNS), including the both olfactory bulbs, nor throughout the entire spinal cord (SC). Rare LNs were found in the central gray matter (CGM) of the midbrain, IRZ of medulla oblongata, and intermediolateral nucleus (IML) of SC. Alzheimer's disease-related neuropathological changes included A0 B1 C0, without phosphorylated TDP-43 (TIP-PTD-P02) proteinopathy. Numerous intraneuritic and intracytoplasmic LBs were found in the stellate ganglia, lower cervical and upper thoracic paravertebral sympathetic trunks, and LNs in the sympathetic nerve fascicles around the aortic arch, coronary arteries, and intermyocardial vessels were immunolabeled by α-synuclein antibodies (5G4, pSyn#64, rabbit polyclonal antibodiesTP-SN-P01 and TIP-SN-P09). In PK-treated sections, immunostaining for these α-synuclein antibodies with pathologically distended structures was observed in the stellate ganglia, lower cervical and upper thoracic paravertebral sympathetic trunks, the sympathetic postganglionic fascicles around the aortic arch, extending to the epicardium, and around the intermyocardial vessels. In sections pre-treated with or without PK, a cocktail of polyclonal α-synuclein antibodies showed more peripheral and thin nerve fibers between the cardiac muscle fibers. No LBs or LNs were found in the submandibular glands, thyroid gland, bronchi, lung (trachea～bronchioles), ENS (esophagus～rectum), adrenal glands, nor abdominal para-aortic sympathetic ganglia. Given the rare LNs in the brain stem and SC, the present case was pathologically characterized as a phenotype of α-synucleinopathy predominantly localized to the cardiac SNS (from the stellate ganglia to the intermyocardial nerves). The presence of similar cases with α-synucleinopathy localized to the cardiac SNS, late-onset parkinsonism with LBs restricted to the DMV, and ILBDs with LBs and LNs predominantly localized to the adrenal gland suggests that α-synucleinopathy occurs independently as a multifocal nervous system disorder. Recently, the various α-synuclein amyloidogenic seed hypotheses were reviewed; to date, the progressive aggregation pathophysiology of α-synuclein through the formation of LBs and LNs remains unknown. The antibody against clone pSyn#64 specifically interacts with α-synuclein possessing a phosphorylated serine 129; clone 5G4 (amino acid 44-57 of α-synuclein) is specific to accumulations of misfolded and pathologically aggregated α-synuclein. Similar to amyloid-β and prion protein, PK-resistant α-synuclein accumulations play a significant role in the pathogenesis of α-synucleinopathy. The pathological findings of the present case suggest that PK-resistant α-synuclein formed throughout the entire PGSN; phosphorylation and aggregation of α-synuclein occurred at the intracytoplasmic and proximal axons of the cardiac sympathetic ganglia associated with the disturbance in axonal transport. Accumulations of cases with localized α-synucleinopathy would settle the problem of 'spreading' routes or 'seeding'. In conclusion, unusual localization of α-synucleinopathy to the cardiac SNS was observed in a patient without neurological manifestations. This focal α-synucleinopathy suggests that PK-resistant α-synuclein developed in the PGSN, where aggregation and phosphorylation of α-synuclein formed LBs and LNs in the intraganglionic cytoplasm and proximal axons. Further studies should be conducted to elucidate the progressive aggregation pathophysi-

ology of α-synuclein through the formation of LBs and LNs.

Publications

Sato S*[1]*, Koike M*[1]*, Funayama M*[1]*, Ezaki J*[2]*, Fukuda T, Ueno T*[1]*, Uchiyama Y*[1]*, Hattori N*[1] *(*[1]Juntendo Univ,*** [2]***Fukusima Med Univ).*** Lysosomal Storage of Subunit c of Mitochondrial ATP Synthase in Brain-Specific Atp13a2-Deficient Mice. *Am J Pathol.* 2016 Dec; **186:** 3074-82.

Sugio S*[1,2]*,Tohyama K*[3]*, Oku S*[1]*, Fujiyoshi K*[5]*, Yoshimura T*[4,5]*, Hikishima K*[4,6]*, Yano R*[4]*, Fukuda T, Nakamura M*[4]*, Okano H*[4]*, Watanabe M*[7]*, Fukata M*[1,2]*, Ikenaka K*[1,2]*, Tanaka KF*[1,4] *(*[1]Natl Inst for Physiol Sci,*** [2]***Schl of Life Sci, SOKENDAI,*** [3]***Iwate Med Univ, Morioka,*** [4]***Keio Univ Schl of Med,*** [5]***Natl Hosp Organi, Murayama Med Ctr,*** [6]***Ctrl Inst for Exp Animals,*** [7]***Hokkaido Univ).*** Astrocyte-Mediated Infantile-Onset Leukoencephalopathy Mouse Model. *Glia.* 2017; **65:** 150-68.

Department of Orthopaedic Surgery
Division of Sports Medicine

Keishi Marumo, *Professor* Hiroki Funasaki, *Associate Professor*

General Summary

Clinical Research
The ongoing research in our department concentrates on competitive athletes (including professional athletes), amateur athletes who include sports activities in their daily activities and young athletes engaged in school sports clubs or dedicated to training within sports clubs. In 2016 we focused mostly on basic research.

Research Activities

Sports activities of middle and older sports enthusiasts who underwent successful conservative treatment for full thickness tear of the rotator cuff
We evaluated sports activities in 54 sports enthusiasts who were older than 40 years and had been conservatively and successfully treated for full thickness tears of the rotator cuff. Their average Japanese Orthopedic Association score (JOA score) was 67 points at initial visits and at the final follow-up it was 89 points; their pain score significantly improved. All patients returned to their previous sport activities. Fifty of 54 patients answered that their treatment was 'more than 80% satisfactory' in regard to their current sports activities. However, post-treatment sport ability depended on tear size, muscle strength, and type of sport activity. In regard to sport disciplines involving the shoulder joint, patients with massive tears and those practicing overhead throws scored worse than other in this group.

Obturator muscle strain in soccer players
We analyzed 9 obturator muscle strain cases in 8 soccer players and examined whether prognosis is related to any specific physical findings, mechanism of injury and/or period from injury to return to play. There were 3 university students and 5 professional players with an average age or 21 years. Strains developed in 7 hips with an obvious mechanism of injury, in 3 hips on the kicking side and in 4 hips on the sustained side. In all players, pain was induced by passive motion of hip abduction and internal rotation. It is important to understand characteristics of physical findings and mechanism of injury for the accurate diagnosis and following prediction of prognosis in obturator muscle strain patients.

Changes in nerve-muscle coordination caused by brain fatigue: An analysis using silent period of quadriceps and hamstrings
We investigated changes of silent period of quadriceps and hamstrings before and after inducing brain fatigue in 11 healthy adults. Fatigue of brain was induced with the Uchida-Kraepelin psycho-diagnostic test. Pre-motion time (PMT) did not show any significant

differences between pre- and post-tolerance test. In contrast, switched silent period (SSP) under brain fatigue condition was significantly prolonged in dominant leg immediately after the tolerance test. Our results suggest that decrease in neuromuscular coordination was induced by brain fatigue.

Changes of gluteus medius muscle activity induced by different joint positions
We measured muscle activities of hip joint muscles during hip abduction using rectified filtered electromyography and investigated changes of theses activities in different joint positions in 14 healthy adults. Percent maximal voluntary contraction (%MVC) of the gluteus medius muscle was obtained by hip abduction with maximal external rotation notwithstanding angle differences of the knee joint. On the other hand, activity of the tensor fasciae latae was lowest in hip abduction with the knee 90 degrees in flexion and with the hip maximal rotation. There was no significant difference in %MCV of the gluteus maximum muscle in different positions of the hip and/or the knee. The results indicated that position for most effective training of the gluteus medius muscle is hip abduction with the hip in maximal external rotation and the knee in 90 degree flexion.

A case of bipartite navicular bone developed in a young soccer player
We reported a rare case of 16-year-old male soccer player who had a bipartite navicular. Care should be taken while making the diagnosis of this disease because it is often detected after trauma and misdiagnosed as an acute fracture and/or fatigue fracture. Although the patient continues with no symptoms to play as a professional soccer player, a long-term careful observation is necessary.

A case of knee osteochondromatosis developed in a nine-year-old girl
We reported a rare case of stage II osteochondromatosis of the knee developed in a 9-year-old girl. She received an arthroscopic resection of pathological synovia and loose bodies. No recurrence was observed at a recent follow-up at one year and 6 months after the operation. There have been only 6 cases reported in the literature where this disease was found in less than 10-year-old patients. In young patients, it is important to discriminate stage I osteochondromatosis for juvenile idiopathic arthritis.

A case of Kocher-Lorenz type capitellar fracture in a junior baseball player occurred due to baseball pitching
We reported a rare case of Kocher-Lorenz type capitellar fracture occurred during baseball pitching of in a 12-year-old baseball player. Although plain X-rays and computed tomogram showed normal findings, MRI demonstrated a 6mm chondral defect at the center of humeral capitulum. Only 3 cases of patients less than 12 years of age who developed Kocher-Lorenz fracture have been reported in the literature and there has been no reports of this fracture that occurred during baseball pitching.

Supraspinatus and subscapularis muscle strains developed in baseball pitchers
Supraspinatus and subscapularis tendon injury are often seen in baseball pitchers. We reported rare two cases of supraspinatus and subscapularis muscle strain (muscle-tendon

junction) developed in high school baseball pitchers. Muscle strains were classified as Grade I in both cases. Following a conservative treatment including physical rehabilitation, both patients were able to return to their previous sports activities.

Publications

Yoshida M, Funasaki H, Marumo K. Efficacy of autologous leukocyte-reduced platelet-rich plasma therapy for patellar tendinopathy in a rat treadmill model. *Muscles Ligaments Tendons J.* 2016; **6:** 205-15.

Department of Pathophysiology and Therapy in Chronic Kidney Disease

Tatsuo Hosoya, *Professor*
Iwao Ohno, *Professor*
Yukio Maruyama, *Assistant Professor*
Satoru Kuriyama, *Professor*
Kimiyoshi Ichida, *Professor*

General Summary

Overview of education and research

This department aims to advance education and research to prevent the onset and development of chronic kidney disease (CKD) and to slow the increase in the number of patients with renal failure. The number of elderly patients undergoing hemodialysis (HD) for renal failure has increased markedly in Japan and has become a critical social and medical economic problem. One solution for this problem is to prevent the onset and progression of CKD and to reduce the number of patients requiring HD.

Another solution is to improve the quality of life for the rehabilitation of patients who have already undergone HD and to promote home HD (HHD) and continuous ambulatory peritoneal dialysis (CAPD) that can be performed at home. Both HHD and CAPD will greatly benefit patients undergoing HD, particularly patients who have difficulty visiting hospitals because of old age or disability. Furthermore, when the Great East Japan Earthquake occurred, it was shown that CAPD could be performed in disaster areas.

Research Activities

Prevention of CKD and its progression

Hyperuricemia has long be suggested to be a risk factor for the onset and progression of CKD, but definitive evidence was lacking, because an antihyperuricemic agent that could reduce uric acid levels effectively and safely in patients with renal dysfunction, such as CKD, was not available. Within the last 3 years, 2 novel antihyperuricemic agents that can be used effectively and safely in patients with renal dysfunction have been developed. The efficacy and safety of one agent, febuxostat, were investigated in patients with CKD IIIb and IV and reported at academic meetings and in a paper. Furthermore, a double-blind multicenter prospective clinical trial (FEATHER study: Febuxostat versus placebo randomized controlled trial regarding reduced renal function in patients with hyperuricemia complicated by chronic kidney disease stage 3), b and the publication is on going.had been conducted in more than 400 patients with CKD IIIa and IIIb by March 2016, and the results will be presented at a conference and published in 2017.

The utility and safety of topiroxostat, another novel antihyperuricemic agent, was investigated in patients with CKD III and hyperuricemia, and its effects on renal function, blood pressure, and albuminuria were examined. The result that albuminuria decreased significantly in patients receiving topiroxostat was reported in a paper. The underlying mechanism of reduced albuminuria is being investigated in basic research, and the effect is

being confirmed separately in a panel of primary diseases for renal failure. Furthermore, a randomized clinical trial to examine the effect of urinaly protein loss caused by diabetic nephropathy is in progress. The results, including a comparison with allopurinol and effects according to type of hyperuricemia, were published.

Efforts to promote CAPD

To promote CAPD, a method of HHD, our department has employed peritoneal dialysis coordinators and had them visit the homes of patients undergoing CAPD to solve the problems presented by the patients and their families. The patients were then asked to answer a questionnaire survey about CAPD; the results were analyzed and presented at academic meetings. Because we believe that HHD by CAPD cannot be promoted without the cooperation of nursing care facilities and health and welfare facilities, CAPD study meetings have been held periodically with colleagues in such facilities near Kashiwa Hospital.

Combination therapy with HD once a week has been tried in patients undergoing CAPD with disturbed peritoneal function or insufficient water removal. A retrospective study and a prospective study (EARTH Study: The study of evaluating adequateness replacement therapy) are ongoing as multicenter collaborative studies to elucidate the effectiveness of the combination therapy. The retrospective study has already been completed and is being prepared for publication. fixed cases and the publication is Registration in the prospective study ended in 2016, and the results will be presented at a conference and published in 2017.

Check-up and evaluation

Research regarding the onset and development of hyperuricemia and CKD is ongoing. The analysis of the FEATHER study ,has been completed in March 2016, and a paper is being made ready for publication.

That topiroxostat reduces albuminuria similarly in a variety of renal diseases has been verified and reported in a paper. Experiments are in progress to elucidate the underlying mechanism in basic studies.

While CAPD has been promoted in patients with renal failure at the Department of Nephrology and Hypertension of our medical school, we hope other institutions will participate in this project and help establish the clinical effecacy of PD and HD combined therapy. To this end, we would like to make proposals for fulfillment of the systems for patients undergoing CAPD, such as medical insurance and nursing care insurance.

Publications

Hosoya T, Sasaki T[1], Ohashi T[1] ([1]Fuji Yakuhin Co., Ltd). Clinical efficacy and safety of topiroxostat in Japanese hyperuricemic patients with or without gout: a randomized, double-blinded, controlled phase 2b study. *Clin Rheumatol.* 2017; **36:** 649-56.

Hosoya T, Ogawa Y[1], Hashimoto H[1], Ohashi T[2], Sakamoto R[1] ([1]Sanwa Kagaku Kenkyusho Co. Ltd., [2]Fuji Yakuhin Co. Ltd). Comparison of topiroxostat and allopurinol in Japanese hyperuricemic patients with or without gout: a phase 3, multicentre, randomized, double-blind, double-dummy, active-controlled, parallel-group study. *J Clin Pharm Ther.* 2016; **41:** 290-7.

Hosoya T, Sasaki T[1], Hashimoto H[2], Sakamoto R[2], Ohashi T[1] ([1]Fuji Yakuhin Co. Ltd., [2]Sanwa

Kagaku Kenkyusho Co. Ltd). Clinical efficacy and safety of topiroxostat in Japanese male hyperuricemic patients with or without gout: an exploratory, phase 2a, multicentre, randomized, double-blind, placebo-controlled study. *J Clin Pharm Ther.* 2016; **41:** 298–305.

Matsuo H[1], Yamamoto K[2], Nakaoka H[3], Nakayama A[1], Sakiyama M[1], Chiba T[1], Takahashi A[4], Nakamura T[1], Nakashima H[1], Takada Y[1], Danjoh I[5,6], Shimizu S[1], Abe J[1], Kawamura Y[1], Terashige S[1], Ogata H[1], Tatsukawa S[1], Yin G[7,8], Okada R[8], Morita E[8], Naito M[8], Tokumasu A[9], Onoue H[1], Iwaya K[1], Ito T[10], Takada T[11], Inoue K[12], Kato Y[13], Nakamura Y[5], Sakurai Y[1], Suzuki H[11], Kanai Y[14], Hosoya T, Hamajima N[15], Inoue I[13], Kubo M[4], Ichida K[12], Ooyama H[8], Shimizu T[15], Shinomiya N[1] ([1]Natl Defense Med Coll, [2]Kurume Univ Sch Med, [3]Natl Institute Genet, [4]RIKEN, [5]RIKEN BioResource Center, [6]Tohoku Univ, [7]Seinan Jo Gakuin Univ, [8]Nagoya Univ Graduate Sch Med, [9]Ryougoku East Gate Clinic, [10]Self-Defense Forces Central Hosp, [11]Univ Tokyo Hosp, [12]Tokyo Univ Pharmacy Life Sci, [13]Kanazawa Univ, [14]Osaka Univ, [15]Midorigaoka Hosp). Genome-wide association study of clinically defined gout identifies multiple risk loci and its association with clinical subtypes. *Ann Rheum Dis.* 2016; **75:** 652–9.

Fujita K[1], Ichida K ([1]Tokyo Univ Pharmacy and Life Sci). A Novel Compound Heterozygous Mutation in the SLC22A12 (URAT1) Gene in a Japanese Patient Associated with Renal Hypouricemia. *Clin Chim Acta.* 2016; **463:** 119–21.

Mancikova A[1], Krylov V[1], Hurba O[1], Sebesta I[1], Nakamura M[2], Ichida K, Stiburkova B[1] ([1]Charles Univ, [2]Tokyo Univ Pharmacy and Life Sci). Functional Analysis of Novel Allelic Variants in URAT1 and GLUT9 Causing Renal Hypouricemia Type 1 and 2. *Clin Exp Nephrol.* 2016; **20:** 578–84.

Matsuo H[1], Tsunoda T[2], Ooyama K[3], Sakiyama M[1], Sogo T[2], Takada T[4], Nakashima A, Nakayama A[1], Kawaguchi M[1], Higashino T[1], Wakai K[5], Ooyama H[3], Hokari R[1], Suzuki H[4], Ichida K, Inui A[2], Fujimori S[6], Shinomiya N[1] ([1]Natl Defense Med Coll, [2]Saiseikai Yokohamashi Tobu Hosp, [3]Ryougoku East Gate Clinic, [4]Univ Tokyo Hosp, [5]Nagoya Univ Graduate Sch Med, [6]Teikyo Univ). Hyperuricemia in Acute Gastroenteritis Is Caused by Decreased Urate Excretion Via ABCG2. *Sci Rep.* 2016; **6:** 31003.

Miyata H[1], Takada T[1], Toyoda Y[1], Matsuo H[2], Ichida K, Suzuki H[1] ([1]Univ Tokyo Hosp, [2]Natl Defense Med Coll). Identification of Febuxostat as a New Strong ABCG2 Inhibitor: Potential Applications and Risks in Clinical Situations. *Front Pharmacol.* 2016; **7:** 518.

Nakayama A[1], Nakaoka H[2], Yamamoto K[3], Sakiyama M[1], Shaukat A[4], Toyoda Y[5], Okada Y[6,7,8], Kamatani Y[7], Nakamura T[1], Takada T[5], Inoue K, Yasujima T[9], Yuasa H[9], Shirahama Y[5], Nakashima H[1], Shimizu S[1], Higashino T[1], Kawamura Y[1], Ogata H[1], Kawaguchi M[1], Ohkawa Y[10], Danjoh I[11], Tokumasu A[12], Ooyama K[12], Ito T[13], Kondo T[14], Wakai K[14], Stiburkova B[15], Pavelka K[15], Stamp KL[16], Dalbeth N[17], Consortium E[18], Sakurai Y[1], Suzuki H[5], Hosoyamada M[19], Fujimori S[20], Yokoo T, Hosoya T, Inoue I[2], Takahashi A[7], Kubo M[7], Ooyama H[12], Shimizu T[21,22], Ichida K, Shinomiya N[1], Merriman TR[5], Matsuo H[1] ([1]Natl Defense Med Coll); Eurogout Consortium. GWAS of Clinically Defined Gout and Subtypes Identifies Multiple Susceptibility Loci That Include Urate Transporter Genes. *Ann Rheum Dis.* 2016; **76:** 869–77.

Okabayashi Y, Yamamoto Y, Komatsuzaki Y, Niikura T, Yamakawa T, Katsumata H, Kawabe M, Katsuma A, Nakada Y, Kobayashi A, Koike Y, Miki J, Yamada H, Tanno Y, Ohkido I, Tsuboi N, Ichida K, Yamamoto H[1], Yokoo Y ([1]Atsugi City Hosp, [2]Natl Institute Genet, [3]Kurume Univ Sch Med, [4]Univ Otago, [5]Univ Tokyo Hosp, [6]Tokyo Med Dental Univ, [7]RIKEN, [8]Osaka Univ Graduate Sch Med, [9]Nagoya City Univ, [10]Kyushu Univ, [11]Tohoku Univ, [12]Ryougoku East Gate Clinic, [13]Self-Defense Forces Central Hosp, [14]Nagoya Univ Graduate Sch Med, [15]Charles Univ, [16]Univ Otago, [17]Univ Auckland, [18]Tokyo Univ Pharmacy Life Sci, [19]Teikyo Univ, [20]Teikyo Univ Sch Med, [21]Midorigaoka Hosp, [22]Kyoto Industrial Health Assoc). Rare Case of Nephrocalcinosis in the Distal Tubules Caused by Hereditary Renal Hypouricaemia 3 Months after Kidney Transplantation. *Nephrology (Carlton).* 2016; **21:** 67–71.

Sakiyama M[1], Matsuo H[1], Nagamori S[2], Ling W[2], Kawamura Y[1], Nakayama A[1], Higashino T[1], Chiba T[1], Ichida K, Kanai Y[2], Shinomiya N[1] ([1]Natl Defense Med Coll, [2]Osaka Univ). Expression of a Human NPT1/SLC17A1 Missense Variant Which Increases Urate Export. *Nucleosides Nucleotides Nucleic Acids.* 2016; **35:** 536–42.

Iguchi A[1], Sato T[1], Yamazaki M[1], Tasaki K[1], Suzuki Y[2], Iino N[3], Hayakawa H[2], Ichida K, Narita I[3] ([1]Saiseikai Niigata Daini Hosp, [2]Tokyo Univ Pharmacy Life Sci, [3]Niigata Univ). A Case of Xanthinuria Type I with a Novel Mutation in Xanthine Dehydrogenase. *CEN Case Rep.* 2016; **5:** 158–62.

Kuriyama S, Nishio S, Kidoguchi S, Honda K, Takahashi Y, Sugano N, Hosoya T, Nakano T, Tanabe T, Stim E, Yokoo T. A Greater Association of Hyperuricemia than of Metabolic Syndrome with the New Incidence of Chronic Kidney Disease. *Open J Nephrol.* 2016; **6:** 17–27.

Morisawa N, Sugano N, Yamakawa T, Kuriyama S, Yokoo T. Successful long-term effect of direct renin inhibitor aliskiren in a patinet with atherosclerotic renovascular hypertension. *CEN Case Rep.* 2017; **6:** 66–73. Epub 2017 Jan 16.

Morisawa N[1], Koshima Y[1], Satoh JI[1], Maruyama Y, Kuriyama S, Yokoo T, Amemiya M[1] ([1]Saitama Red Cross Hospital). Usefulness of combination therapy with Daclatasvir plus Asunaprevir in chronic hepatitis C patients with chronic kidney disease. *Clin Exp Nephrol.* Epub 2016 Oct 22.

Department of Innovative Interventional Endoscopy Research

Hisao Tajiri, *Professor* Masato Mitsunaga, *Assistant Professor*

General Summary

This department was established in April, 2015, aiming at methodology of new endoscopic diagnosis and treatment, and also the development of apparatus, along with for the purpose of supporting and teaching to arrange the environment toward the standardization of endoscopic medicine not only in domestic but also in foreign facilities.

Research Activities

Endoscopic submucosal resection (ESD), which was developed in Japan, is followed by various improvements to conduct safely, promptly and accurately. Subsequently to ESD, new minimally invasive endoscopic treatments, such as endoscopic full thickness resection and endoscopic treatment applying robotic technology are being developed one after another. Now Japan is reaching an aging society at unprecedented speed among other countries, while its population is decreasing. Japanese world-class technology cultivated from experience of craftsmanship is a base for the development of endoscopy and even more growth is expected in the future. Especially minimally invasive endoscopic treatments with less burden to patients contribute significantly. While social demand for endoscopic medicine is growing, it is meaningful to propel new methodology for endoscopic treatment and development of instruments for it.
This department plays a role, in addition to the study of the above, to support formulation of educational structure of endoscopy for the doctors not only in Japan but in Asia, Russia, Middle East, and South America.

Development of supporting devices for ESD and clinical evaluation
One of the problems of the oral and transanal endoscopic therapy such as ESD is that only the skilled doctors can conduct them safely. Existing surgical instruments are electric scalpels, piercing through the forceps at its entrance of about 2.8 mm in diameter to penetrate a flexible endoscope, which move only to-and-fro. It makes extremely difficult to lift an affected part and cut the inside open while operating a fiberscope minutely with all these devices. The endoscopic system, which can operate by two hands of right and left to move freely, has been expected for long time, and an elasticity forceps to bend have been researched and developed all over the world. But they are not practical since the current smallest ones were 4mm in diameter so far, not being able to insert in the forceps channel (2.8 mm) of the existing elasticity endoscope. An article specially made to order costs expensive. Since a flexible endoscope is expensive, it is a medical condition to make ends meet economically to develop one which can be used daily. Because a forcep's outlet of flexible endoscope commercially available is around 3 mm in diameter, a flexible forcep

of around 2.6 mm in diameter is necessary to insert. The flexible flexure forceps of 2.6 mm in diameter was succeeded (Nakadate R et al. Endoscopy 2015; 47(9): 820–4). This promoted appearance of medical device for practical use which may make ends meet economically. Furthermore, in consideration for utility and economy, two control sticks are equipped to the fixed base to be manipulated stable, and a grip of flexible scope and its console are placed as can be reached at the same time so one endoscopist can conduct procedure. The flexible endoscopes can be removed anytime for manual manipulation when it is necessary. Besides they are robotic devices not requiring motors, which make them as nearest as practical application. We have been repeating animal studies of in vivo, ex vivo and evaluated their clinical usefulness. We will continue to work on the technological development for conducting ESD without stress.

Endoscopic optical molecular imaging for cancer

Molecular targeted therapies, such as monoclonal antibodies, were widely used for various cancers recently, leading to improve patients' outcomes. Use of the molecular targeted medicine for cancer patients generally depend on the level of molecular expression in the targeted tumor, therefore, developing a method of companion diagnosis is required at the same time of developing a molecular-targeted therapy. We have developed a method of molecular target-specific fluorescence cancer imaging and phototherapy, called photoimmunotherapy. To expand the applicability of photoimmunotherapy, we developed a novel photoactivatable bifunctional antibody-drug conjugate that can work as both photoimmunotherapy and chemoimmunotherapy agents. We evaluated the feasibility of IR700 conjugated trastuzumab emtansine (T-DM1-IR700)-mediated near-infrared light irradiation by comparing the in vitro and in vivo cytotoxic efficacy of trastuzumab-IR700 (T-IR700)-mediated NIR light irradiation in HER2-expressing cells. In vivo photoimmunotherapy using T-DM1-IR700 did not show a superior antitumor effect to photoimmunotherapy using T-IR700 in subcutaneous small-tumor models, which could receive sufficient near-infreared light. In contrast, photoimmunotherapy using T-DM1-IR700 tended to reduce the tumor volume and showed significant prolonged survival compared to photoimmunotherapy using T-IR700 in large-tumor models that could not receive sufficient near-infrared light.

Currently, phase 2 clinical study is ongoing for recurrent head and neck cancer patients in multiple US sites. This method could serve as an aid for expanding the indication of photoimmunotherapy.

Publications

Goda K, Dobashi A, Yoshimura N, Aihara H, Kato M, Sumiyama K, Toyoizumi H, Kato T, Saijo H, Ikegami M, Tajiri H. Dual-focus versus conventional magnification endoscopy for the diagnosis of superficial squamous neoplasms in the pharynx and esophagus: a randomized trial. *Endoscopy.* 2016; **48:** 321–9.

Muto M, Yao K, Kaise M, Kato M, Ueda N, Yagi K, Tajiri H. Magnifying Endoscopy Simple Diagnostic Algorithm for Gastric Cancer (MESDA-G). *Dig Endosc.* 2016; **28:** 379–93.

Yoshikawa K[1], Kurihara C[1], Furuhashi H[1], Takajo T[1], Maruta K[1], Yasutake Y[1], Sato H[1], Narimatsu K[1], Okada Y[1], Higashiyama M[1], Watanabe C[1], Komoto S[1], Tomita K[1], Nagao S[1], Miura S[1], Tajiri H, Hokari R[1] ([1]Natl Defense Med Coll). Psychological stress exacerbates NSAID-induced small bowel injury by inducing

changes in intestinal microbiota and permeability via glucocorticoid receptor signaling. *J Gastroenterol.* 2017; **52:** 61-71.

Hosoe N[1], Watanabe K[2], Miyazaki T[2], Shimatani M[3], Wakamatsu T[3], Okazaki K[3], Esaki M[4], Matsumoto T[5], Abe T[1], Kanai T[1], Ohtsuka K[4], Watanabe M[4], Ikeda K, Tajiri H, Ohmiya N[6], Nakamura M[7], Goto H[7], Tsujikawa T[8], Ogata H[1] ([1]Keio Univ, [2]Osaka City Univ Graduate Sch Med, [3]Kansai Med Univ, [4]Tokyo Med Dental Univ, [5]Iwate Med Univ, [6]Fujita Health Univ, [7]Nagoya Graduate Sch Med, [8]Shiga Univ Med Sci). Evaluation of the performance of the Omni mode for detecting video capsule endoscopy images: A multicenter randomized controlled trial. *Endosc Int Open.* 2016; **4:** E878-82.

Takamaru H[1], Yamada M[1], Sakamoto T[1], Nakajima T[1], Saito Y[1], Kakugawa Y[1], Matsumoto M[1], Matsuda T[1], Ide D, Gulati S[2], Tajiri H ([1]Natl Cancer Center, [2]King's Coll Hosp). Dual camera colon capsule endoscopy increases detection of colorectal lesions. *Scand J Gastroenterol.* 2016; **51:** 1532-3.

Kawamura T[1], Oda Y[2], Kobayashi K[3], Matsuda K[4], Kida M[3], Tanaka K[1], Kawahara Y, Yokota I[5], Koizumi W[3], Yasuda K[1], Tajiri H ([1]Kyoto Second Red Cross Hosp, [2]Oda GI Endoscop Gastroenterol Clinic, [3]Kitasato Univ, [4]St. Marianna Univ Sch Med, [5]Kyoto Prefectural Univ Med). Colonoscopy withdrawal time and adenoma detection rate: a Japanese multicenter analysis. *J Gastroenterol Hepatol Res.* 2017; **6:** 1-6.

Reviews and Books

Murdani A[1], Kumar A[2], Chiu HM[3], Goh KL[4], Jang BI[5], Khor CJL[6], Lau J[7], Mostafa I[8], Ramchandani M[9], Ratanalert S[10], Tajiri H, Yuen M[11], Zhanq ST[12], Duforest-Rey D[13], Rey JF[13] ([1]Universitas Indonesia, [2]Fortis Escorts Liver and Digestive Diseases Institute, [3]Natl Taiwan Univ Hosp, [4]Univ Malaya, [5]Yeungnam Univ Coll Med, [6]Singapore General Hosp, [7]The Chinese Univ Hong Kong, [8]Theodor Bilharz Research Institute, [9]Asian Institute of Gastroenterology, [10]NKC Institute of Gastroenterology, Songklanagarind Hospital, [11]Cantel Medical Asia/Pacific Pte. Ltd, [12]Beijing Friendship Hospital, [13]Institut Arnault Tzanck). WEO position statement on hygiene in digestive endoscopy: Focus on endoscopy units in Asia and the Middle East. *Dig Endosc.* 2017; **29:** 3-15.

Miyazaki R, Arihiro S, Hayashi E, Kitahara T, Oki S, Kamba S, Ide D, Komoike N, Satoh K, Saruta M, Kato T, Tajiri H, Aoki H, Omura N, Mitsumori N, Mitsuishi T, Yanagisawa H, Takahashi H. A giant gastrointestinal stromal tumor of the stomach with extramural growth. *Case Rep Gastroenterol.* 2016; **10:** 344-51.

Department of Innovation for Medical Information Technology

Hiroyuki Takao, *Associate Professor and Director*

General Summary

This course deals broadly with ICT (Information and Communication Technology), an area that has seen remarkable development in recent years, including everything from basic research on its development to clinical application, with the aim of using ICT in medical care.
We are studying the development of wearable devices and AI (artificial intelligence) that link with telecommunications. We are also conducting research and development toward implementing ICT medical care in a wide variety of areas, including health management, emergency care sites, intra-hospital networks, and chronic-phase rehabilitation and nursing care.

Research Activities

Research and development of a communication application for medical personnel
We are researching and developing a piece of software called "Join," the first such software to be covered by insurance in Japan. The research investigates factors including the cost-effectiveness provided by communication in the field of stroke treatment, in which the time leading up to diagnosis and treatment is especially important.

Research and development of a health support application
We are researching and developing a piece of software called "MySOS." When an emergency arises, this app seeks help from nearby people, and helps make the decision whether or not to go to a hospital, referring to emergency manuals for adults and children. Future development will focus on enabling linkage with hospitals.

IoT (Internet of Things) development (checking blood pressure by smartphone, etc.)
We are going forward with the development of IoT wearable devices as a means of accumulating large quantities of data. In the development of wristwatch-type blood pressure meters and band-type EEGs, we are advancing development from the standpoint of storing large amounts of personal medical information in the cloud via smartphone, and defending against illness.

Mobile phone electromagnetic wave effects
We are doing research relating to the effects of smartphones on medical equipment. The research will determine whether there really are no issues with using smartphones at medical care facilities. We are publishing a paper on this subject.

Medical equipment development (intracranial stents, etc.)
We are conducting discussions on the development of medical equipment, as well as the practical development of intracranial stents. Currently, the Japanese medical equipment industry is heavily dependent on imports. Our ultimate goal is to contribute to the advancement of the domestic health care industry by offering various types of support and holding actual physician-led clinical trials, so that the health care industry in Japan can be self-sufficient.

Introducing ICT medical care
We are doing various studies on the introduction of ICT medical care. It is said that using ICT in various aspects of nursing and caregiving would improve work efficiency in these areas. The aim is to put this into practice.

Medical results of using robots
We are conducting research, using Pepper, on interaction between robots and people. We are studying what changes occur in health care facilities when people see and come into contact with robots.

Publications

Takao H, Yu Y, Arita H, Oobatake T, Sakano T, Kurihara M, Matsuki A, Ishibashi T, Murayama Y. Primary salvage survey of the interference of radio waves emitted by smartphones on medical equipment. *Health Phys.* 2016; **111:** 381-92.

Research Center for Medical Sciences
Division of Gene Therapy

Toya Ohashi, *Professor and Director* Hiroshi Kobayashi, *Associate Professor*

General Summary

We mainly studied gene therapy for lysosomal storage disease such as mucopolysaccharidosis type II, Krabbe diease and Fabry disease, using various viral vector. In addition, we also developing small molecule therapy for Pompe disease and mucopolysaccharidosis type II. Regarding cancer, we studied anticancer effect of nafamostat mesilate, a serine-protease inhibitor, for various type of cancer. Our main mission is to translate these observation to clinic.

Research Activities

Gene therapy for lysosomal storage disease using lentivirus vector and genome editing
We investigated the bone system in mucopolysaccharidosis type II (MPS II), and detected increasing of bone mass, trabecular bone, bone density, and bone strength comparing to normal group. We generated lentiviral vector expressing iduronate-2-sulfatese and carried out hematopoietic stem cell targeted *ex-vivo* gene therapy for MPS II model mouse. The improvement of bone involvement was observed. And we also investigated the effect of newborn gene therapy, combination with substrate reduction therapy (SRT), and *in vitro* gene editing (using Zinc Finger system) for Krabbe disease model mouse.

Development of novel therapy for Pompe disease by using proteasome inhibitor
We evaluated the bortezomib-induced blood toxicity in murine model of Pompe disease. As a result, no significant difference was observed in the number of both leukocytes and platelets between Pompe mice treated with or without bortezomib. This result suggests that bortezomib may exert a positive effect on α-glucosidase activity in Pompe mice without induction of blood toxicity.

Effect of sulfated disaccharides on mutated IDS in Mucopolysaccharidosis type II
We investigated the effect of sulfated disaccharides on mutated iduronate-2-sulfatase (IDS) by using cell-based assay. Sulfated disaccharides improved the enzyme activity of several types of mutated IDS which have an amino acid substitution around of active site.

Improvement of peripheral neuropathy in Fabry disease mouse by AAV9 vector
Fabry disease (FD) is a genetic disorder caused by mutation of the *GLA* gene, resulting in accumulation of globotriaosyl ceramide (Gb3) in various tissues including dorsal root ganglia (DRG). FD patients have peripheral neuropathy from childhoods. We injected rAAV9 encoding hGLA (rAAV2/9-hGLA) intrathecally (i.t.) to reduce the Gb3 accumulation in DRG. The GLA enzyme activity in the lumber DRG of rAAV-FD mice was

increased compare to wild type mice. FD mice showed a thermal hypoalgesia in hot-plate test, and the level of thermal hypoalgesia in the rAAV-FD mice recovered to the level of wild type mice. As a conclusion, the i.t. administration of rAAV2/9-hGLA transduced neural cells of DRG and improves the peripheral neuropathy of FD.

Enhancement of antitumor effect by NF-κB inhibitor for digestive cancers and treatment of cancer pain

We have reported that nafamostat mesilate, a serine-protease inhibitor, inhibits NF-κB activation and enhances anti-tumor effects for pancreatic cancer. The clinical usefulness of the combination chemotherapy of gemcitabine with nacamostat mesilate was examined in phase II study. Moreover, thalidomide, the first generation of IMiDs, has anti-tumor effects for myeloma and colorectal cancer cells. Recently, standard chemotherapies for unresectable pancreatic cancer are gemcitabine/S-1 or gemcitabine/nab-paclitaxel, thus we investigated combination chemotherapy of these agents with IMiDs. Ionizing radiation enhances epithelial-mesenchymal transition (EMT) and cancer metastasis. We examined whether NF-κB inhibitor suppresses EMT in chemoradiation for colorectal cancer. Cancer pain affects QOL of patients with cancer. We are investigating the mechanism of cancer pain and a new treatment strategy.

Publications

Shirai Y, Uwagawa T, Shiba H, Shimada Y, Horiuchi T, Saito N, Furukawa K, Ohashi T, Yanaga K. Recombinant thrombomodulin suppresses tumor growth of pancreatic cancer by blocking thrombin-induced PAR1 and NF-κB activation. *Surgery.* 2017 Jun; **161:** 1675-82.

Matsui M, Inoue K, Sakoda S, Matsumura T, Hamazaki T, Kosuga M, Okuyama T, Shimada Y, Ohashi T, Nishino I. Screening for late-onset Pompe disease in undiagnosed myopathies. *Neur Clin Neurosci.* 2017; **5:** 60-4.

Horiuchi T, Uwagawa T, Shirai Y, Saito N, Iwase R, Haruki K, Shiba H, Ohashi T, Yanaga K. New treatment strategy with nuclear factor-κB inhibitor for pancreatic cancer. *J Surg Res.* 2016 Nov; **206:** 1-8.

Kono Y, Wakabayashi T, Kobayashi M, Ohashi T, Eto Y, Ida H, Iguchi Y. Characteristics of cerebral microbleeds in patients with Fabry Disease. *J Stroke Cerebrovasc Dis.* 2016 Jun; **25:** 1320-5.

Yokoi T, Yokoi K, Akiyama K, Higuchi T, Shimada Y, Kobayashi H, Sato T, Ohteki T, Otsu M, Nakauchi H, Ida H, Ohashi T. Non-myeloablative preconditioning with ACK2 (anti-c-kit antibody) is efficient in bone marrow transplantation for murine models of mucopolysaccharidosis type II. *Mol Genet Metab.* 2016 Nov; **119:** 232-8.

Sato Y, Kobayashi H, Higuchi T, Shimada Y, Ida H, Ohashi T. TFEB overexpression promotes glycogen clearance of Pompe disease iPSC-derived skeletal muscle. *Mol Ther Methods Clin Dev.* 2016 Aug 10; **3:** 16054.

Sato Y, Kobayashi H, Higuchi T, Shimada Y, Ida H, Ohashi T. Metabolomic profiling of pompe disease-induced pluripotent stem cell-derived cardiomyocytes reveals that oxidative stress Is associated with cardiac and skeletal muscle pathology. *Stem Cells Transl Med*. Epub 2016 Aug 18. pii: sctm. 2015-0409.

Limkul J, Iizuka S, Sato Y, Misaki R, Ohashi T, Ohashi T, Fujiyama K. The production of human glucocerebrosidase in glyco-engineered Nicotiana benthamiana plants. *Plant Biotechnol J.* 2016 Aug; **14:** 1682-94.

Hughes DA, Nicholls K, Shankar SP, Sunder-Plassmann G, Koeller D, Koeller K, Vockley G, Hamazaki T, Lachmann R, Ohashi T, Olivotto I, Sakai, N, Deegan P, Dimmock D, Eyskens F, Germain DP, Goker-Alpan O, Hachulla E, Jovanovic, A, Lourenco CM, Narita I, Thomas M, Wilcox WR, Bichet DG, Schiffmann R, Ludington E, Viereck C, Kirk J, Yu J, Johnson F, Boudes P, Benjamin ER, Lockhart DJ, Barlow, C, Skuban N, Castelli JP, Barth J, Feldt-Rasmussen U. Oral pharmacological chaperone migalastat compared with enzyme replacement therapy in Fabry disease: 18-month results from the randomised phase III ATTRACT study. *J Med Genet.* 2017 Apr; **54:** 288-96.

Kopajtich R, Murayama K, Janecke AR, Haack TB, Breuer M, Knisely AS, Harting I, Ohashi T, Okazaki Y, Watanabe D, Tokuzawa Y, Kotzaeridou U, Kölker S, Sauer S, Carl M, Straub S, Entenmann A, Gizewski E, Feicht-

inger RG, Mayr JA, Lackner K, Strom TM, Meitinger T, Müller T, Ohtake A, Hoffmann GF, Prokisch H, Staufner C. Biallelic IARS mutations cause growth retardation with prenatal onset, intellectual disability, muscular hypotonia, and infantile hepatopathy. *Am J Hum Genet.* 2016 Aug; **99:** 414–22.

Kato S, Yabe H, Takakura H, Mugishima H, Ishige M, Tanaka A, Kato K, Yoshida N, Adachi S, Sakai N, Hashii Y, Ohashi T, Sasahara Y, Suzuki Y, Tabuchi K. Hematopoietic stem cell transplantation for inborn errors of metabolism: A report from the Research Committee on Transplantation for Inborn Errors of Metabolism of the Japanese Ministry of Health, Labour and Welfare and the Working Group of the Japan Society for Hematopoietic Cell Transplantation. *Pediatr Transplant.* 2016 Mar; **20:** 203–14.

Shimada Y, Wakabayashi T, Akiyama K, Hoshina H, Higuchi T, Kobayashi H, Eto Y, Ida H, Ohashi T. A method for measuring disease-specific iduronic acid from the non-reducing end of glycosaminoglycan in mucopolysaccharidosis type II mice. *Mol Genet Metab.* 2016; **117:** 140–3.

Higuchi T, Kobayashi M, Ogata J, Kaneshiro E, Shimada Y, Kobayashi H, Eto Y, Maeda S, Ohtake A, Ida H, Ohashi T. Identification of cryptic novel α-galactosidase A gene mutations: abnormal mRNA splicing and large deletions. *JIMD Rep.* 2016; **30:** 63–72.

Research Center for Medical Sciences
Division of Oncology

Sadamu Homma, *Professor and Director*
Masaki Ito, *Assistant Professor*
Shigeo Koido, *Associate Professor*
Yasuharu Akasaki, *Assistant Professor*

General Summary

The aim of our researches is to develop and establish novel cancer therapies. Concepts of new anti-cancer therapy generated from unique idea of the researchers would be verified by basic and clinical studies in order to apply such concepts to the clinical cancer treatment. Most of our researches have been based on antitumor immunity.

Research Activities

Comprehensive analyses on gene expression of human glioblastoma mutiforme (GM) for search of the predictive biomarkers for clinical effectiveness of dendritic cell (DC) therapy

DC vaccine for post-operative prevention or treatment against GM was generated by cell fusion of DCs and GM cells derived from the individual patients. The next generation sequencer for this investigation was TorrentSuite (ThermoFisher) and the transcripts of the GM cells used for the generation of DC vaccine were listed. The results demonstrated that neutrophil-associated genes such as chemokines or cytokines were highly expressed in GM cells from non-effective cases compared with those from effective cases. It has been known that neutrophils suppress the activity of cytotoxic T lymphocytes which play a central role in antitumor immunity. In non-effective case, antitumor T cells induced by DC therapy might have been inactivated by neutrophils recruited in tumor microenvironment by neutrophil-associated genes expressed in GM cells. Neutrophil-associated gene expression in GM cells could become biomarkers for prediction of the effectiveness of the DC therapy against GM.

Claudin 7 as a novel molecular target for treatment of pancreatic cancer

From a human pancreatic cancer cell line, MIA PaCa-2, MIA PaCa-2-A cells with epithelial morphology and MIA PaCa-2-R cells with non-epithelial morphology were clonogenically isolated by the limiting dilution method. Although the MIA PaCa-2-A and MIA PaCa-2-R cells displayed the same DNA short tandem repeat (STR) pattern identical to that of the parental MIA PaCa-2 cells, the MIA PaCa-2-A cells were more proliferative than the MIA PaCa-2-R cells both in culture and in xenografts generated in SCID mice. Furthermore, the MIA PaCa-2-A cells were more resistant to gemcitabine than the MIA PaCa-2-R cells. DNA microarray analysis demonstrated high expression of Claudin (CLDN) 7 in the MIA PaCa-2-A cells but not in the MIA PaCa-2-R cells. Knockdown of CLDN7 in the MIA PaCa-2-A cells using siRNA induced marked inhibition of proliferation without altering the cell morphology. The MIA PaCa-2-A cells with CLDN7 knock-

down showed G1 cell cycle arrest. CLDN7 might be expressed in the rapidly proliferating and dominant cell population in human pancreatic cancer tissues and might be a novel molecular target for the treatment of pancreatic cancer.

High PD-L1 expression indicates poor prognosis of HIV-infected non-small cell lung cancer patients

The status of antitumor immunity represented by the expression of programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1) and the immune cell (IC) infiltration was unknown in HIV-infected non-small cell lung cancer (NSCLC) patients. Fifteen HIV-infected and 29 non-HIV-infected, or 13 of each propensity score matched NSCLC patients were analyzed. The expression of PD-1/PD-L1 and the infiltration of $CD4^+$, $CD8^+$ and $CD56^+$ ICs were examined by immunohistochemistry, and score ≥ 2 was defined as positive. In analysis of all the patients as well as the propensity matched cohort, high PD-L1 expression group showed shorter survival than low PD-L1 expression group in HIV-infected patients, whereas significant difference of survival was not observed between high and low PD-L1 expression groups of non-HIV cohort. High PD-L1 expression in tumor tissue clearly indicated poor prognosis in HIV-infected NSCLC patients but not in non-HIV-infected NSCLC patients. These results suggest that suppression of antitumor immunity by PD-1/PD-L1 axis might be stronger in HIV-infected NSCLC patients than in non-HIV-infected NSCLC patients.

Significance of functional soluble programmed cell death ligand-1 (sPD-L1) in blood of advanced cancer patients

We have previously reported that the plasma levels of sPD-L1 in patients with advanced pancreatic cancer (PC) were higher than those of healthy subjects. Although PD-L1 is expressed in PC tissues, PD-L1 expression in tumor tissue and plasma sPD-L1 level were not correlated. Furthermore, plasma sPD-L1 levels were not associated with serum CA19-9 levels or tumor sizes in PC patients, indicating that main source of sPD-L1 is not tumor tissue. Plasma sPD-L1 levels declined along with the decrease in blood lymphocyte count with progression of the disease. We found that monocyte derived dendritic cells (DCs) from PC patients released abundant sPD-L1 *in vitro* but not non-adherent cells in peripheral blood mononuclear cells. sPD-L1 from DCs could activate PD-1 signaling in the cell-based functional assay for PD-1/PD-L1 interaction, suggesting that sPD-L1 is functionally immunosuppressive. sPD-L1 in cancer patients should be involved in the suppression of antitumor immunity and become a novel target for the next generation cancer immunotherapy.

A functional cell-based assay for immune checkpoint molecules

Immune checkpoint inhibitors such as the antibodies targeting the programmed death 1 (PD-1) receptor have shown promising results in multiple cancers. However, it has been shown that the expression level of programmed death-1 ligand-1 (PD-L1) in tumor does not necessarily correlate with the therapeutic effect of anti-PD-1 antibody. PD-1 contains two immmunoreceptor tyrosine-based motifs (ITIM and ITAM) that are phosphorylated upon receptor engagement and recruit Src homology 2-domain-containinng tyrosine

phosphatase 2 (SHP2). We developed the functional cell-based assay system to detect the recruitment of SHP2 to PD-1 by the interaction of PD-1 and its ligands PD-L1 in the TCR-independent condition. Split bioluminescence reporters were used for detecting the interactions between PD-1 and SHP-2. Antigen presenting cells expressing PD-L1 and PD-L2 elicited strong PD-1 signaling in this assay system, but the PD-1 signal intensity induced by tumor cells did not correlate with the PD-L1 expression level of tumor cells. Our assay system representing a functional PD-1 signaling status may be useful in searching for factors influencing the PD-1 signaling pathway.

Publications

Hayashi K, Nagasaki E, Kan S, Ito M, Kamata Y, Homma S, Aiba K. Gemcitabine enhances rituximab-mediated complement-dependent cytotoxicity to B cell lymphoma by CD20 upregulation. *Cancer Sci.* 2016; **107:** 682-9.

Akasaki Y, Kikuchi T, Homma S, Koido S, Ohkusa T, Tasaki T, Hayashi K, Komita H, Watanabe N, Suzuki Y, Yamamoto Y, Mori R, Arai T, Tanaka T, Joki T, Yanagisawa T, Murayama Y. Phase I/II trial of combination of temozolomide chemotherapy and immunotherapy with fusions of dendritic and glioma cells in patients with glioblastoma. *Cancer Immunol Immunother.* 2016; **65:** 1499-509.

Chiba M, Imazu H, Kato M, Ikeda K, Arakawa H, Kato T, Sumiyama K, Homma S. Novel quantitative analysis of the S100P protein combined with endoscopic ultrasound-guided fine needle aspiration cytology in the diagnosis of pancreatic adenocarcinoma. *Oncol Rep.* 2017; **37:** 1943-52.

Okuma Y[1], Hosomi Y[1], Nakahara Y[1], Watanabe K[1] ([1]Tokyo Metropolitan Hospital), Sagawa Y, Homma S. High plasma levels of soluble programmed cell death ligand 1 are prognostic for reduced survival in advanced lung cancer. *Lung Cancer.* 2017; **104:** 1-6.

Reviews and Books

Okamoto M[1], Kobayashi M[1] ([1]Kitasato U), Yonemitsu Y[2] ([2]kyusyu U), Koido S, Homma S. Dendritic cell-based vaccine for pancreatic cancer in Japan. *World J Gastrointest Pharmacol Ther.* 2016 Feb 6; **7:** 133-8.

Research Center for Medical Sciences
Division of Molecular Immunology

Saburo Saito, *Associate Professor and Director*
Nobutake Akiyama, *Assistant Professor*
Daitaro Kurosaka, *Professor*
Yuji Ohno, *Assistant Professor*

General Summary

Our research interests have focused on the analysis of the basic immune system, which protects us from a number of diseases, and of immune disorders, such as hypersensitivity diseases and autoimmune diseases.

Research Activities

Regulation of Th2 responses by different cell types expressing the interleukin-31 receptor
Interleukin-31 (IL-31) is a recently identified cytokine produced by Th2 cells that is involved in the development of atopic dermatitis-induced skin inflammation and pruritus. Its receptor, IL-31RA, is expressed by a number of cell types, including epithelial cells, eosinophils, and activated monocytes and macrophages. To date, however, the regulation of Th2 responses by distinct cell types and tissues expressing IL-31RA has not been well studied.
In this study, Cry j 2, one of the major allergens of Japanese cedar pollen, was administered to IL-31RA-deficient or wild-type (WT) mice via nasal or intraperitoneal injection for induction of specific Th2 responses.
After nasal administration of Cry j 2, IL-31RA-deficient mice showed lower Cry j 2-specific CD4+ T cell proliferation, Th2 cytokine (IL-5 and IL-13) production, and Th2-mediated (IgE, IgG1, and IgG2b) antibody responses than WT mice. In contrast, IL-31RA-deficient mice administered Cry j 2 intraperitoneally showed stronger Th2 immune responses than WT mice.
These results indicate that IL-31R signaling positively regulates Th2 responses induced by nasal administration of Cry j 2, but negatively regulates these responses when Cry j 2 is administered intraperitoneally. Collectively, these data indicate that the induction of antigen-specific Th2 immune responses might depend on tissue-specific cell types expressing IL-31RA.

Evaluation of allergen-specific immune responses induced by oral immunotherapy with transgenic rice containing major T-cell epitopes of Japanese cedar pollen allergens in patients with cedar pollinosis
Oral immunotherapy with dominant T-cell epitopes is safer and more effective than conventional immunotherapy for the treatment of immunoglobulin E-mediated allergic diseases. In the previous study, a blinded, randomized, placebo-controlled trial employing oral immunotherapy with 80 g of steamed pack rice for cedar pollinosis was performed for 20 weeks. Thus, oral administration of the rice was found to be a safe therapy without

side effects. The aim of this study was to investigate whether oral immunotherapy with small dose of the transgenic rice seed is effective to induce oral tolerance in patients with Japanese cedar pollinosis. Double blinded, randomized, placebo-controlled trial employing oral immunotherapy with 5 g or 20 g of steamed pack rice for cedar pollinosis was performed for 8 weeks. Twenty-one subjects were enrolled and divided into 3 groups that ate 5 g or 20 g of transgenic rice or normal rice.

No major adverse effects were observed in either group during treatment. Allergen-specific T-cell responses were evaluated. The ratio of allergen-specific T cells proliferative responses to 7Crp peptide, Cry j 1, and Cry j 2 were significantly lower in subjects who ate transgenic rice than in subjects who ate normal rice. Furthermore, allergen-driven IL-5 and IL-13 were also significantly reduced in culture supernatants of peripheral blood mononuclear cells after subjects had eaten transgenic rice. Taken together, oral immunotherapy with small dose of the transgenic rice was expected to be an effective treatment for cedar pollinosis.

Current clinical studies are being conducted to evaluate the clinical efficacy of oral immunotherapy with small dose of the transgenic rice.

Adjuvant for inducing antigen-specific cytotoxic T lymphocytes via cross-presentation of cationic lipids

Vaccine that raises specific cytotoxic T cells against tumors or pathogens is the convincing approach to overwhelm these diseases. By the past study, we have developed a new liposome based adjuvant to induce CTL by just mixing protein antigens and adjuvant before the administration. After administration with antigens having some kind of protein structure and adjuvant, inductions of antigen specific CTL by cross priming were observed. Then anti-tumor activities were measured by vacctinations with this adjuvant and melanoma cell extract. As a result, the growth and metastasis of melanomas were significantly inhibited. At present, we are developing the methods to induce specific CTLs against other kind of tumors by vaccination.

Publications

Ito H, ***Noda K***, ***Yoshida K***, ***Otani K***, ***Yoshiga M***, ***Oto Y***, ***(Saito S)***, ***Kurosaka D.*** Prokineticin 2 antagonist, PKRA7 suppresses arthritis in mice with collagen-induced arthritis. *BMC Musculoskelet Disord.* 2016; **17:** 387.

Research Center for Medical Sciences
Division of Medical Engineering

Masayuki Yokoyama, *Professor and Director* Kouichi Shiraishi, *Assistant Professor*

General Summary

The division of Medical Engineering provides new and essential techniques for developments of medical treatment. We have developed a new concept for an acute ischemic stroke treatment by the use of polymeric micelle drug carrier systems. For this project, we have collaborated closely with clinical departments and basic science laboratories, both in our university and hospitals and others. In acute phase of ischemic stroke, recombinant human tissue-type plasminogen activator (rt-PA) is an only therapeutic drug for the thrombolysis therapy. However, there are a number of reports that rt-PA accelerates a risk of hemorrhage. For safety treatment of rt-PA therapy, a novel diagnostic concept to reduce the hemorrhage risk is highly desired. We have developed a novel approach for diagnosis of acute ischemic stroke and have assessed the risk of hemorrhage in rt-PA therapy. We have applied a polymeric micelle magnetic resonance imaging (MRI) contrast agent to assess the risk of hemorrhage. We have examined fundamental study of poly(ethylene glycol) (PEG)-related immunogenicity. PEG is the most popular polymer for pharmaceutics, cosmetics, and foods. PEGs are known to exhibit very weak immunogenicity, however, immunogenicity of PEGylated drugs has become a serious concern for PEGylated therapeutic drugs. We revealed the reason of PEG-related immunogenicity and found that PEG-related immunogenicity is controllable. We further examined fundamental study of PEG-related immunogenicity to reveal responses of T-cell independent antigens.

Research Activities

Assessment of hyper-permeable blood-brain barriers (BBBs) in ischemic stroke

In acute ischemic stroke, rt-PA is an only drug for the thrombolytic treatment. However, there are a number of reports that rt-PA accelerates a risk of hemorrhage. For safety use of rt-PA, a novel diagnostic concept is highly desired. We have developed polymeric micelle carrier systems, and the polymeric micelle carrier systems are nano-sized drug carriers which capable of carrying therapeutic drugs and diagnostic drugs. We are trying to develop the next generation of novel treatment based on the drug carrier systems. We have applied the polymeric micelle MRI contrast agent system to hyper-permeable BBB in a rat acute ischemic stroke model and had obtained high contrast images in the tissues. We started to examine molecular weight (MW)-dependent hyper-permeability in BBB. To assess the hyper-permeable BBB, we prepared two different MW of poly(glutamic acid) (P(Glu))-based MRI contrast agents. To examine BBB's MW-dependent hyper-permeability, we used a rat transient middle cerebral artery occlusion (MCAO)-reperfusion model. In a 3-hour MCAO-reperfusion model, we injected P(Glu)-based MRI contrast

agents at immediately after reperfusion. The injected dose was 0.033 mmol Gd/kg which is one third dose of the clinical dose. Firstly, we examined 30k MW P(Glu)-based MRI contrast agent. However, we obtained very faint contrast in the tissues, whereas we succeeded in the MCAO model. This was probably owing to short plasma half-life of the 30k MW P(Glu)-based MRI contrast agent, as well as a rapid clearance rate of the 30k MW P(Glu)-based MRI contrast agent from the tissues. Next, we examined 100k MW P(Glu)-based MRI contrast agent. This P(Glu)-based MRI contrast agent exhibited high contrast in the tissues in a short time period. In contrast, we found that the obtained contrast became low at 3 h after reperfusion.

One serious concern for a development of MRI contrast agent is toxicity of gadolinium ion. Free gadolinium ions released from gadolinium-chelate complexes are deposited on tissues, as a result, insoluble gadolinium precipitate caused nephrogenic systemic fibrosis (NSF). To avoid such release of free gadolinium ions, we must use a very stable gadolinium-chelate complex as a MRI contrast agent. We have been studying polymer MRI contrast agents, however, one carboxylic group of a chelate group was used for a conjugation to polymer backbone. Therefore, gadolinium-chelate complexes, which was conjugated to polymers, were not extremely stable. For preparation of the stable gadolinium-chelate complex, we started to use a novel chelate for gadolinium ions for P(Glu)-based MRI contrast agent. We are trying to evaluate stability of a P(Glu)-based novel gadolinium-chelate complex, as compared with previously prepared P(Glu)-based MRI contrast agents.

Polymeric micelle drug carrier systems in immune system

We have been studying immunogenicity of poly(ethylene glycol) (PEG). PEG has been widely used for drug carriers, as well as protein drugs. However, generation of antibodies against PEG (anti-PEG antibodies) have become serious issues in the PEGylated proteins-treated patients. Repeatedly injected PEGylated proteins induced anti-PEG antibodies, and efficacy of therapy was lost. These PEGylated protein-treated patients exhibited anti-PEG IgG and anti-PEG IgM. Furthermore, there are reports that PEG-liposomes induced specific IgM antibody against PEG (anti-PEG IgM). We confirmed that PEG-poly(β-benzyl L-aspartate) block copolymer (PEG-PBLA) induced anti-PEG IgM. Although PEG has been known to show no or very weak immunogenicity, the phenomenon exhibits a specific immune response against PEG. We revealed that PEG is an essential part to exhibit PEG-specific antibody responses, however, PEG itself does not exhibit the PEG-specific antibody response. We concluded that this was owing to PEG possessing no strong binding affinity. Very unique characteristics of PEG motivate us to examine further study of PEG-related immunogenicity, and we found fundamental insights in immune response system. We repeatedly injected PEG-PBLA, as an antigen, to induce antibody against PEG and found that PEG-PBLA induced anti-PEG IgG, as well as anti-PEG IgM. We only found anti-PEG IgG responses at very low dose PEG-PBLA as the first dose, and the anti-PEG IgG response was not observed when high dose PEG-PBLA was injected at the first dose. We performed further experiments to examine anti-PEG IgG responses. We found that switch Ig class to IgG occurred when the anti-PEG IgM response exhibited low responses, while switch Ig class did not occur when the anti-PEG

IgM response exhibited high responses. We concluded that PEG-related antibody responses are a dose dependent response, and high affinity antigens need low doses to induce antibody responses. Therefore, in general, we observe IgM responses by high affinity T-cell independent antigens, but this response does not exclude IgG responses.

Publications

Shiraishi K, Kawano K[1], Maitani Y[1], Aoshi T[2,3], Ishii KJ[2,3], Sanada Y[4], Mochizuki S[4], Sakurai K[4], Yokoyama M ([1]Hoshi Univ, [2]Osaka Univ, [3]National Institute of Biomedical Innovation, [4]Kitakyushu Univ). Exploring the relationship between anti-PEG IgM behaviors and PEGylated nanoparticles and its significance for accelerated blood clearance. *J Control Release.* 2016; **234:** 59-67.

Wang Z, Sawaguchi Y[1], Hirose H[2], Ohara K[2], Sakamoto[2] ([1]Nihonyakka Univ, [2]Kaneka Corp), Mitsumura H, Ogawa T, Iguchi Y, Yokoyama M. An In vitro assay for sonothrombolysis based on the spectrophotometric measurement of clot thickness. *J Ultrasound.* 2017; **36:** 681-98.

Wang Z, Komatsu T, Mitsumura H, Nakata N, Ogawa T, Iguchi Y, Yokoyama M. An uncovered risk factor of sonothrombolysis: Substantial fluctuation of ultrasound transmittance through the human skull. *Ultrasonics.* 2017; **77:** 168-75.

Shiraishi K, Wang Z, Kokuryo D[1], Aoki I[1] ([1]National Institutes for Quantum and Radiological Science and Technology), Yokoyama M. polymeric micelle magnetic resonance imaging (MRI) contrast agent reveals blood-brain barrier (BBB) permeability for macromolecules in cerebral ischemia-reperfusion injury. *J Control Release.* 2017 May 10; **253:** 165-71. Epun 2017 Mar 18.

Research Center for Medical Sciences
Division of Ultrasound Device Development and Application

Norio Nakata, *Associate Professor and Director* Zuojun Wang, *Assistant Professor*

Research Activities

Development of ultrasonic prevention of vascular occlusion
Reocclusion occurred after various recanalization therapies. In particular, reocclusion was frequently observed immediately after the recombinant tissue-type plasminogen activator (rt-PA) treatment. This is a fatal problem, because anticoagulant therapy is prohibited within 24 hours after the rt-PA treatment. We report the thrombus growth control effect of non-invasive ultrasound (US) in an in vitro clot growth model. In this study, we showed that the non-invasive US could control the growth of thrombus. This safe and simple US method may be used to prevent the reocclusion after recanalization therapy.

Study of leveling of transcranial ultrasound transmittance
Transcranial ultrasound thrombolysis promotion therapy for acute stage cerebral infarction is being researched and developed. In this therapy, ultrasonic cranial bone permeability is an important factor regulating effectiveness and safety. In this research, we are studying ultrasonic modulation technology which greatly fluctuates the transmittance and reduces the fluctuation.

Development of decision supporting system of breast ultrasound using deep learning
The purpose of this study is to develop decision supporting system of breast ultrasonography using deep learning which is one of machine learning techniques. The goal of this system is a classification tool between benign and malignant breast mass lesions. For this study, at least 1,000 cases of supervised data sets including breast ultrasound images and pathologic diagnosis results are required. This study has already approved by the Jikei University Ethics Committee. We are preparing the installation of the deep learning program and the experiment of AI tests. Improvement of diagnostic efficiency of breast ultrasound diagnostic radiologists is expected by this study.

Education and awareness activities to promote AI utilization in diagnostic radiology
From 12th January to 29th March 2017, a roundtable discussion meeting on promotion of AI utilization in the health care field was held at the Ministry of Health, Labor and Welfare four times in total. Director Nakata participated as a member from this research department and deepened the discussion about the current situation and future prospects for utilization of AI in healthcare policy in Japan in diagnostic imaging, and summarized the recommendations as a report
(Reference: http://www.mhlw.go.jp/stf/shingi/other-kousei.html?tid=408914).

Publications

Wang Z, Komatsu T, Mitsumura H, Nakata N, Ogawa T, Iguchi Y, Yokoyama M. An uncovered risk factor of sonothrombolysis: Substantial fluctuation of ultrasound transmittance through the human skull. *Ultrasonics.* 2017; **77:** 168-75.

Wang Z, Sawaguchi Y, Hirose H, Ohara K, Sakamoto S, Mitsumura H, Ogawa T, Iguchi Y, Yokoyama M. An In Vitro Assay for Sonothrombolysis Based on the Spectrophotometric Measurement of Clot Thickness. *J Ultrasound Med.* 2017; **36:** 681-98. Epub 2017 Feb 2.

Sawaguchi Y, Wang Z. Ultrasound Acceleration of rt-PA Thrombolysis Depends on Acoustic Intensity. *Biol Pharm Bull.* 2017; **40:** 97-103.

Shiraishi K, Wang Z, Kokuryo D, Aoki I, Yokoyama M. A polymeric micelle magnetic resonance imaging (MRI) contrast agent reveals blood-brain barrier (BBB) permeability for macromolecules in cerebral ischemia-reperfusion injury. *J Control Release.* 2017; **253:** 165-71. Epub 2017 Mar 18.

Yokoyama M, Wang Z, Nakata N, Mimura H, Iguchi Y. Leveling the transcranial ultrasound transmittance. *Ultrasonic Technology.* 2016; **28:** 24-7.

Research Center for Medical Sciences
Division of Neuroscience

Fusao Kato, *Professor and Director* Ayako M. Watabe, *Associate Professor*

General Summary

The integration and coordination of functions throughout the body is realized mainly through intercommunication via the nervous systems. To understand how the activities of organs affect brain activity and, in turn, how the brain controls the activities of organs to optimize these integrative functions, we must clarify the mechanisms underlying the dynamic cell-to-cell signaling in the central nervous system underlying various specific functions, such as pain and emotion. In particular, plastic changes of the central nervous system "wiring" realized through the variability of synaptic connections in response to various environmental changes form the core mechanism for optimizing human and animal behaviors. In addition, such plastic changes are known to underlie psychosomatic pathological states, such as chronic pain without sustained tissue injury or inflammation. We use approaches at the molecular, cellular, and network levels, including the patch-clamp recording of synaptic currents, the real-time imaging of the intracellular Ca^{2+} concentration, and optogenetic approaches to activate a specific set of neurons by light in living brain tissues from normal animals, animal models of various diseases, and animals subjected to experimental manipulation of gene expression and combine them with the detailed analysis of the behavior of these animals.

Research Activities

Central mechanisms underlying chronic pain

Lines of evidence indicate the establishment of chronic pain involves plastic changes in the "pain matrix" in the central nervous system playing roles in sensation, emotion and cognition of pain. We analyzed the cellular and network mechanisms underlying this process.

1. We demonstrated that the monosynaptic inputs from the parabrachial nucleus to the central amygdala not only activates the central amygdala neurons but also gives rise to sustained post-excitation inhibition using selective activation of these inputs using optogenetics with channel rhodospin-expression systems.

2. We created rats expressing cre recombinase under promotor activities for dopa-β-hydroxylase (DBH) and vesicular GABA transpoter (VGAT). Using these rats, we have demonstrated that pharmacogenetical excitation of central amygdala neurons with DREADD (designer receptor exclusively activated by designer drug) expression technique results in hyperalgesia and their suppression in attenuated nocifensive behaviors in inflammatory pain model. Also using these rats with channelrhodopsin2 expression, we have directly measured the synaptic transmission from the central amygdala to the periaqueductal grey neurons, which had been otherwise impossible. These results provide basis

for understanding the role of descending pain modulation system, through which the brain controls the nociception sensitivity especially in the chronic pain.
3. We have applied small animal magnetic resonance imaging with an ultrahigh magnetic field scanner to visualize the spontaneous cerebral activities with activity-dependent Mn^{2+} uptake during the establishment of chronic pain. We found that during the development of inflammatory chronic pain, widely distributed brain areas, such as the limbic systems, are strongly activated.
4. We have demonstrated in mice that an artificial suppression of the amygdala neurons which had been activated commonly in distinct memory forming tasks, using activity-dependent expression of archeorhodopsin and its optogenetic stimulation, reduces behaviors depending on associative memories.

Mechanism underlying motor neuron vulnerability
In motor neuron degenerative diseases such as the amyotrophic lateral sclerosis (ALS), the vulnerability of the neurons against metabolic stress differs between distinct motor neuron pools. To elucidate the mechanism underlying such region-dependent vulnerability, we compared the responses to experimental chemical hypoxia in brain slices between hypoglossal, facial and oculomotor neurons. In the hypoglossal and facial motor neurons, which are highly vulnerable in ALS, glycine release was increased. In contrast, in the oculomotor neurons, which are most resistant in the ALS, GABA release was increased instead. Because we have already demonstrated that an increase in extracellular glycine concentration leads to enhanced excitotoxity through activation of NMDA receptors, this particularity would underlie the distinct vulnerability of different motor neurons.

Publications

Takagi S, Kono Y, Nagase M, Mochio S, Kato F. Facilitation of distinct inhibitory synaptic inputs by chemical anoxia in neurons in the oculomotor, facial and hypoglossal motor nuclei of the rat. *Exp Neurol.* 2017; **290:** 95–105. Epub 2017 Jan 19.

Tsurugizawa T, Takahashi Y, Kato F. Distinct effects of isoflurane on basal BOLD signals in tissue/vascular microstructures in rats. *Sci Rep.* 2016; **6:** 38977.

Nomoto M, Ohkawa N, Nishizono H, Yokose J, Suzuki A, Matsuo M, Tsujimura S, Takahashi Y, Nagase M, Watabe AM, Kato F, Inokuchi K. Cellular tagging as a neural network mechanism for behavioural tagging. *Nat Commun.* 2016; **7:** 12319.

Sugimura YK, Takahashi Y, Watabe AM, Kato F. Synaptic and network consequences of monosynaptic nociceptive inputs of parabrachial nucleus origin in the central amygdala. *J Neurophysiol.* 2016; **115:** 2721–39.

Research Center for Medical Sciences
Division of Clinical Pharmacology and Therapeutics

Shigeru Kageyama, *Professor and Director* Akihiro Ohnishi, *Professor*

General Summary

The Division of Clinical Pharmacology and Therapeutics was established in July 1995. The aim of the division is to investigate drug treatment, mainly in the area of internal medicine, whereas other departments of clinical pharmacology in Japan focus on registration trials, particularly phase I trials. Because a clinical laboratory where we had performed many human pharmacological studies became unavailable in 2003, we shifted our research from human studies to multicenter clinical trials and pharmacoepidemiological studies.

Research Activities

So far, we have performed a large-scale pharmacoepidemiological study on the safety of statins. It took a quite a long time to complete it, therefore, we organized a research group comprising academic and industrial organizations (Japanese Society for Pharmacoepidemiology, Japanese Society of Clinical Pharmacology and Therapeutics, Japan Association for Medical Informatics, Japan Society of Clinical Trials and Research, Federation of Pharmaceutical Manufacturer's Associations of Japan, Pharmaceutical Research and Manufacturers of America, and European Federation of Pharmaceutical Industries and Associations Japan) to make postmarketing studies more efficient by utilizing the Standardized Structured Medical-record Information eXchange (SS-MIX). The SS-MIX system was started in 2006 as a project supported by the Ministry of Health, Labour and Welfare for promoting the exchange of standardized medical information. The SS-MIX system will increase the efficiency of pharmacoepidemiological studies by identifying "new users" who started the drug after some period of nonuse. The "new user" design is often essential for unbiased results. In the 3 Jikei University Hospitals (Katsushika Medical Center, Daisan Hospital, and Kashiwa Hospital) where electronic medical record systems have already installed we are going to collect prescription and medical test data to make diabetic disease registries. We are planning to broaden disease registries to various diseases.

To raise the literacy of clinical trials among researchers we held "Clinical Trial Seminar" 2 times this year. The themes were as follows: "Systems for improving the quality of clinical trials." (May 2016), "Basic knowledge on intellectual property for researchers." (March 2017).
An administrative office for registration trials was established in the hospital in February 1999, and the system for registration trials in the hospital has been reformed to meet the demands of the new good clinical practice guidelines. Ten clinical research coordinators

(CRCs) now facilitate clinical trials of which 2 CRCs have mainly been involved in monitoring. The CRCs have started to help with both registration trials and investigator-initiated trials. The CRCs have been introduced into all registration trials since 2004; the quality and speed of these trials were much improved.
As the introduction of CRCs into investigator-initiated trials increased, we invited CRCs from site management organizations to supplement CRCs involved in registration trials.

Publications

Odawara M*[1]*, Kawamori R*[2]*, Tajima N, Iwamoto Y*[3]*, Kageyama S, Yodo Y*[4]*, Ueki F*[4]*, Hotta N*[5] *(*[1]Tokyo Medical Univ,*** [2]***Juntendo Univ Grad Sch Med,*** [3]***Asahi Life Foundation,*** [4]***Sumitomo Dainippon Pharma,*** [5]***Chubu Rosai Hosp).***

Long-term treatment study of global standard dose metformin in Japanese patients with type 2 diabetes mellitus. *Diabetology Int.* 2017; **8:** 286-95. Epub 2017 Feb 24.

Research Center for Medical Sciences
Division of Molecular Epidemiology

Mitsuyoshi Urashima, *Professor and Director*

General Summary

Despite having the same disease diagnosis, some patients may be cured but some may not. This difference cannot be understood with experimental medicine. On the other hand, clinical practice might also not provide the answer. We combined molecular biology and epidemiology to create the Division of Molecular Epidemiology, to clarify the etiology of disease and to predict factors affecting survival.

Research Activities

The Jikei clinical research course

We held 20 seminars' about strategies for clinical studies for healthcare practitioners at The Jikei University. In 2015, small-group study courses targeting postgraduate students will be started from the principles of epidemiology and biostatistics by reading textbooks and by analyzing real clinical data with STATA software (StataCorp LP, College Station, TX, USA) and designing clinical studies. Our goal is for postgraduate students to develop the skills to construct hypotheses, design protocols, monitor trials, and analyze data.

Original studies

1. Randomized trial of vitamin D supplement
2. Elective class of Global Health
3. Randomized trial to prevent food allergy

Publications

Niwa S, Mezawa H, Kobayashi N, Ida H, Urashima M. Inverse association between maternal 25OHD level and cord GLP-1/GIP concentrations. *Pediatr Res.* 2016 Apr; **79:** 536-42.

Martineau AR[1], Cates CJ[2], Urashima M, Jensen M[3], Griffiths AP[1], Nurmatov U[4], Sheikh A[5], Griffiths CJ[1] ([1]Queen Mary Univ, [2]Univ London, [3]Univ Newcastle, [4]Cardiff Univ, [5]Univ Edinburgh). Vitamin D for the management of asthma. *Cochrane Database Syst Rev.* 2016 Sep 5; **9:** CD011511.

Ishikawa Y, Tanaka H, Akutsu T, Koide K, Sakuma M, Okazaki M, Ida H, Urashima M. Prenatal vitamin A supplementation associated with adverse child behavior at 3 years in a prospective birth cohort in Japan. *Pediatr Int.* 2016 Sep; **58:** 855-61.

Mafune A, Iwamoto T, Tsutsumi Y, Nakashima A, Yamamoto I, Yokoyama K, Yokoo T, Urashima M. Associations among serum trimethylamine-N-oxide (TMAO) levels, kidney function and infarcted coronary artery number in patients undergoing cardiovascular surgery: a cross-sectional study. *Clin Exp Nephrol.* 2016 Oct; **20:** 731-9.

Shimura E, Hama T, Suda T, Ikegami M, Urashima M, Kojima H. The Presence of HPV DNA in Neck Lymph Node Metastasis Correlates with Improved Overall Survival of Patients with Oropharyngeal Cancer Undergoing Surgical Treatment. *Oncology.* 2017; **92:** 87-93. Epub 2016 Dec 1.

Martineau AR[1], Jolliffe DA[1], Hooper RL[1], Greenberg L[1], Aloia JF[2], Bergman P[3], Dubnov-Raz G[4], Esposito S[5], Ganmaa D[6], Ginde AA[7], Goodall EC[8], Grant CC[9], Griffiths CJ[1],

Janssens W*[10]*, Laaksi I*[11]*, Manaseki-Holland S*[12]*, Mauger D*[13]*, Murdoch DR*[14]*, Neale R*[15]*, Rees JR*[16]*, Simpson S Jr*[17]*, Stelmach I*[18]*, Kumar GT*[19]*, Urashima M, Camargo CA Jr*[20] *([1]Queen Mary Univ, [2]Winthrop Univ Hosp, [3]Karolinska Institutet, [4]Edmond and Lily Safra Children's Hosp, [5]Univ Milan, [6]Harvard Sch Public Health, [7]Univ Colorado Sch Med, [8]McMaster Univ, [9]Univ Auckland, [10]Universitair ziekenhuis Leuven, [11]Univ Tampere, [12]Unive Birmingham, [13]The Pennsylvania State Univ, [14]Univ Otago, [15]QIMR Berghofer Medical Research Institute, [16]Geisel Sch Medicine at Dartmouth, [17]Univ Tasmania, [18]Med Univ Lodz, [19]Univ Delhi, [20]Massachusetts General Hosp). Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ.* 2017 Feb 15; **356:** i6583.

Research Center for Medical Sciences
Division of Clinical Epidemiology

Masato Matsushima, *Professor and Director*

General Summary

Division of Clinical Epidemiology is promoting the activity of clinical research, clinical epidemiology and education concerning them. Our specific aim is to support clinicians to solve their own problems in daily practice by epidemiological/clinical research skills.
The research themes of our division are medical communication, quality assessment of medical care, behavioral medicine, outcome research, qualitative research as well as disease-oriented epidemiological research. In particular, we aim to produce evidence in the field of primary-care due to the lack of evidence although primary-care is a front-line of practice.
As a contribution to the undergraduate education, our division holds classes of "Evidence-based clinical practice (EBCP)" to make medical students a skillful doctor being able to employ evidence-based approach.
Our postgraduate education concentrates on the methodology of clinical/epidemiological research and biostatistics. "The educational program for primary-care on clinical research methodology", which was started in 2007 by financial support of the Ministry of Education, Culture, Sports, Science and Technology in Japan was renewed as "Jikei Clinical Research Program for Primary-care" in 2009. Furthermore, as a subprogram of the project, "New Paradigms-Establishing Centers for Fostering Medical Researchers of the Future" supported financially by the Ministry of Education, Culture, Sports, Science and Technology in Japan, "Community Health and Primary Care Medicine" in the doctoral course was launched in 2014. The main aim of these programs is to make a primary-care physician a clinician-researcher.

Research Activities

EMPOWER-JAPAN study: Elderly Mortality Patients Observed Within the Existing Residence

Little is known concerning the prognosis of patients receiving home medical care in Japan. EMPOWER-JAPAN study was started as a multi-centered prospective cohort study to mainly describe in-home mortality and clarify its predictors. The cohort consisted of patients who have been newly introduced to home medical care at more than 10 teaching-clinics in Tokyo, Kanagawa, and Saitama. The follow-up period was until January 31st, 2017. This study was financially supported by Japan Society for the Promotion of Science.

Comparison of diabetes care between specialists and general practitioners by the chronic care model

The chronic care model was developed during 1990's in the United States to improve the

care of chronic illness by refining care-provider system, especially in a primary-care setting. The aim of the study was to compare the quality of diabetes care between specialists in diabetes and primary-care physicians as non-specialists by using the official Japanese version of the assessment form "Assessment of Chronic Illness Care".

Cohort study of patient's complexity
As the size of the aged population increases, the patients' complexity on biomedical and psychosocial issues is thought to also increase. The aim of the study was to examine the effect of patients' complexity on length of stay in hospital by employing the "Patient Centred Assessment Method".

Development of Japanese version of Patient Centred Assessment Method
We are developing the Japanese version of "Patient Centred Assessment Method" that evaluates the patient complexity. The process of translation, back-translation and verification by original authors has been done. As the next step, we plan to do pre-test (cognitive debriefing).

Ecology of medical care on an isolated island
The retrospective open cohort study in Iheya, an isolated island in Okinawa Prefecture was performed to describe the ecology of medical care. Due to the free-access medical system in Japan people can have access to advanced-care-medical facility without a referral letter by primary care physician. Thus, it is quite difficult to evaluate the gate keeping function by primary care clinic in Japan. To accomplish it, the frequencies of visits to the clinic in the island and referrals to medical facilities outside the island were calculated, and the comparison between the previous nationwide survey and our study was made.

Publications

Watanabe T, Matsushima M, Nagata T, Tominaga T, Yokoyama H (Jiyugaoka Medical Clinic), Fujinuma Y (Center for Family Medicine Development, Japanese Health and Welfare Co-operative Federation). Evaluation of the Diabetes Chronic-care System in Japanese Clinics. *Jikeikai Med J.* 2016; **63:** 63-70.

Dobashi A, Goda K, Yoshimura N, Ohya TR, Kato M, Sumiyama K, Matsushima M, Hirooka S, Ikegami M, Tajiri H. Simplified criteria for diagnosing superficial esophageal squamous neoplasms using Narrow Band Imaging magnifying endoscopy. *World J Gastroenterol.* 2016 Nov 7; **22:** 9196-204.

Kaneko M, Matsushima M, Irving G (University of Cambridge). The ecology of medical care on an isolated island in Okinawa, Japan: a retrospective open cohort study. *BMC Health Serv Res.* 2017 Jan 14; **17:** 37.

Wakabayashi H (Yokohama City University Medical Center), Matsushima M, Ichikawa H, Murayama S, Yoshida S, Kaneko M, Mutai R. Occlusal support, dysphagia, malnutrition, and activities of daily living in aged individuals needing long-term care: A path analysis. *J Nutr Health Aging.* Epub 2017 Mar 1.

Research Center for Medical Sciences
Division of Regenerative Medicine

Hirotaka James Okano, *Professor and Director*

General Summary

Regenerative medicine is rapidly moving toward translation to clinical medicine. However, a better understanding of the molecular pathways that lead to human diseases is required for regenerative medicine to succeed. Good animal models will play a key role in studies leading to a greater understanding of the pathophysiology of neurodegenerative diseases. On the other hand, induced pluripotent stem cell (iPSC) technology has allowed us to generate and expand various types of differentiated cell from patient-derived cells; these differentiated cells can be applied to cell therapy and to the study of the mechanisms of disease in human cells. Advances in disease modeling using patient-derived cells and primates will have huge effects on future opportunities and progress in biomedical research.

Research Activities

Disease-related RNA binding proteins

Diagnostic biomarkers for amyotrophic lateral sclerosis (ALS) have yet to be identified. One of the causes of neuronal cell death in neurodegenerative diseases is abnormal RNA metabolism, although the mechanisms by which this occurs are unclear. Detection of abnormal RNA metabolism in white blood cells (WBCs) could lead to a new biomarker of ALS onset. TAR DNA-binding protein 43 kDa (TDP-43) is an RNA-binding protein that regulates RNA metabolism. We previously developed a mouse model of ALS that exhibits adult-onset motor dysfunction; these mutant TDP-43 knock in (KI) mice heterozygously express mutant human TDP-43 (A382T or G348C). In the present study, we examined TDP-43 mRNA levels in WBCs of KI mice and found that A382T mutant mRNA is significantly higher than G348C. Our results suggest that each mutant TDP-43 induces distinct RNA metabolism, and that the expression of total TDP-43 alone in WBC is not suitable as an ALS biomarker. To identify additional candidates, we focused on survival and apoptosis-related factors and examined their mRNA metabolism in WBCs. mRNA levels of both Smn1 and Naip5 correlated with TDP-43 levels and also differed between A382T and G348C. Together, TDP-43 and these factors may enable detection of abnormalities in individual ALS pathologies (Hasegawa M. et al. *Neurosci Res*. 2016).

In vivo modeling of human diseases

During rodent experiments, the caudal ventral artery (CVA) is useful for blood pressure (BP) measurement. However, CVA measurements may not reflect the true BP. This study was performed to verify the site-specific accuracy of invasive arterial BP monitoring during surgery in rats. Invasive arterial BP was simultaneously measured in rats via the CVA

and the common carotid artery (CCA). The BP values were analysed while the rats were subjected to cooling of the head or tail. Additionally, the rats underwent digital subtraction angiography and histological examination of these arteries. The pressure difference was more significant in the tail cooling group than in the head cooling group. Digital subtraction angiography revealed that angiospasms occurred more frequently in the CVA than in the CCA upon cooling. This phenomenon was supported by histological analysis, which showed that the tunica media area was significantly larger in the CVA than in the CCA. CVA pressure is susceptible to environmental changes and may not accurately reflect the true BP without a strictly controlled laboratory environment. Therefore, understanding the pitfalls of this method is necessary to avoid cooling of the tail during BP measurement (Ohta H. et al. *Sci Rep.* 2017).

fMRI was conducted to investigate allodynia in mice; allodynia was generated by surgical injury at the L4 spinal nerve root, thus selectively stimulating sensory nerve fibers. In intact mice, only the primary somatosensory cortex (S1) was activated by stimulation of Aβ-fibers. Meanwhile, allodynic mice showed significantly higher BOLD signals in the anterior cingulate area (ACA) and thalamus. Using resting state fMRI, both degree and eigenvector centrality were significantly decreased in the contralateral S1, clustering coefficient and local efficiency were significantly increased in the ACA, and betweenness centrality was significantly higher in the ventral posterolateral nucleus of the thalamus. These results suggest that the observed abnormal BOLD activation is associated with defects in Aβ-fibers when Aβ-fibers in allodynic mice are selectively stimulated（Komaki Y. et al. *Sci Rep.* 2016).

Effects of high-energy particles on neural activity in cortical neurons

"*Light flashes" (LF),* are phosphenes reported by most astronauts in space missions outside the magnetosphere of the Earth. The conditions of occurrence have been thought retinal effects of cosmic ray including heavy ions or protons. A small fraction of the LF might be caused by Cherenkov radiation, while the majority is probably caused by some kind of direct interaction with elements in the retina, as some reports suggest LF could also be sensations of light produced by the activation of neurons along the visual pathway. However cell biological mechanisms of LF have not been addressed yet.

A research group of National Institute of Radiological Sciences (NIRS) in Japan constructed a microbeam facility (named as SPICE) by using our HVEE Tandem accelerator (3.4 MeV proton) and they have shown various biological effects of microbeam irradiation with protons in culture cells. This imaging system, with the position resolution less than 2μm, enables to perform Ca imaging of a single cultured neuron that attach down the bottom of a culture dish. Intracellular calcium is a second messenger that plays important roles in regulating many cell functions and Ca imaging allows real-time analyses of individual cells.

To develop an *in vitro* experimental system, we irradiated cultured mouse cortical neurons with repeated bursts of protons by SPICE and observed neuronal activities by Ca imaging before and after irradiation. The proton irradiation evoked calcium responses in an irradiated single neuron and also it seemed to inhibit the spontaneous calcium oscillation in surrounding cells, which might be a bystander effect of irradiation.

In spaceflight, especially in long travels like Mars mission, the astronauts' brain is exposed to cosmic ray including many protons, from which we are normally protected by the Earth's magnetic shield. Understanding the cell biological bases of the effects of protons to the brain function will be a priority issue in preparations of manned deep space missions.

Publications

Katsuoka Y, Ohta H, Fujimoto E, Izuhara L, Yokote S, Kurihara S, Yamanaka S, Tajiri S, Chikaraish T[1], Okano HJ, Yokoo T ([1]St. Mrianna Univ). Intra-arterial catheter system to repeatedly deliver mesenchymal stem cells in a rat renal failure model. *Clin Exp Nephrol.* 2016; **20:** 169-77.

Kobayashi R[1], Takahashi-Fujigasaki J, Shiozawa S[1], Hara-Miyauchi C, Inoue T[2], Sasaki E[2], Okano HJ, Okano H[1] ([1]Keio Univ, [2]CiEA). α-synuclein aggregation in the olfactory bulb of middle-aged common marmoset. *Neurosci Res.* 2016; **106:** 55-61.

Hasegawa M, Hara-Miyauchi C, Ohta H, Sakimura K[1], Okano H[2], Okano HJ ([1]Niigata Univ, [2]Keio Univ). Analysis of RNA metabolism in peripheral WBCs of TDP-43 KI mice identifies novel biomarkers of ALS. *Neurosci Res.* 2016; **106:** 12-22.

Itoh M, Kawagoe S, Okano HJ, Nakagawa H. Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a patient with Lymphedema-Distichiasis Syndrome (LDS) carrying an insertion-deletion complex mutation in the FOXC2 gene. *Stem Cell Res.* 2016; **16:** 611-3.

Itoh M, Kawagoe S, Okano HJ, Nakagawa H. Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a healthy individual: WT-iPSC1. *Stem Cell Res.* 2016; **17:** 22-4.

Itoh M, Kawagoe S, Okano HJ, Nakagawa H. Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a healthy individual: WT-iPSC2. *Stem Cell Res.* 2016; **17:** 16-8.

Itoh M, Kawagoe S, Okano HJ, Nakagawa H. Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a healthy individual: WT-iPSC4. *Stem Cell Res.* 2016; **17:** 19-21.

Itoh M, Kawagoe S, Okano HJ, Nakagawa H. Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a patient with recessive dystrophic epidermolysis bullosa (RDEB) carrying two compound heterozygous mutations in the COL7A1 gene. *Stem Cell Res.* 2016; **17:** 32-5.

Inagaki Y[1], Fujioka M[1], Kanzaki S[1], Watanabe K[1], Oishi N[1], Itakura G[1], Yasuda A[1], Shibata S[1], Nakamura M[1], Okano HJ, Okano H[1], Ogawa K[1] ([1]Keio Univ). Sustained effect of hyaluronic acid in subcutaneous administration to the cochlear spiral ganglion. *PLoS One.* 2016; **11:** e0153957.

Komaki Y[1], Hikishima K[1], Shibata S[1], Konomi T[1], Seki F[1], Yamada M[2], Miyasaka N[3], Fujiyoshi K[1], Okano HJ, Nakamura M[1], Okano H[1] ([1]Keio Univ, [2]Fujita Health Univ, [3]TMDU). Functional brain mapping using specific sensory-circuit stimulation and a theoretical graph network analysis in mice with neuropathic allodynia. *Sci Rep.* 2016; **6:** 37802.

Fujimoto E, Yamanaka S, Kurihara S, Tajiri S, Izuhara L, Katsuoka Y, Yokote S, Matsumoto K, Kobayashi E, Okano HJ, Chikaraish T[1], Yokoo T ([1]St. Mrianna Univ). Embryonic Kidney Function in a Chronic R 1 enal-failure Model in Rodents. *Clin Exp Nephrol.* 2017 Aug; **21:** 579-588. Epub 2016 Sep 16.

Ohta H, Ohki T, Kaneoka Y, Koizumi M, Okano HJ. Pitfalls of invasive blood pressure monitoring using the caudal ventral artery in rats. *Sci Rep.* 2017; **7:** 41907.

Hikishima K[1], Komaki Y[1], Seki F[1], Ohnishi Y[2], Okano HJ, Okano H[1] ([1]Keio Univ, [2]CiEA). *In vivo* microscopic voxel-based morphometry with a brain template to characterize strain-specific structures in the mouse brain. *Sci Rep.* 2017; **7:** 85.

Research Center for Medical Sciences
Core Research Facilities for Basic Science
(Division of Molecular Genetics)

Hisashi Yamada, *Professor and Director*
Yumi Kanegae, *Associate Professor*
Tsuyoshi Kashima, *Assistant Professor*

General Summary

We can now analyze a person's whole genome. These technological developments have started a new era of medicine. The etiology and therapy of disease will be studied on the basis of genetics. As physicians of today, our research fields are the epigenetic control of cancers and neurodegenerative disorders. Gene therapy has become an attractive procedure to cure diseases. We contribute to gene therapy through the development of regulation of gene-expression and genome editing.
Our division plays a role in supporting various research studies. We served more than 8,000 sequence analysis. The management of the cell sorter and the next-generation sequencer were satisfactory.

Research Activities

Molecular pharmacology of anticancer agents
Ample evidences indicate that epigenetic dysfunctions play an important role in leukemogenesis. We previously showed that targeting bromodomain and extra-terminal (BET) family proteins had shown therapeutic efficacy in diverse hematologic malignancies and solid cancers. However, treatment with BET inhibitors induces various resistance responses, the resistance mechanism remains poorly understood. We established I-BET151-resistant U937 cells (U937R) and compared the characterization of these cells. Treatment with I-BET151 induced a growth inhibition and apoptosis in U937 cells, but not in U937R cells. The drug sensitivity test showed that IKK inhibitor VII had significant higher sensitivity in U937R cells than in U937 cells. BRD2, BRD4, and nuclear NFkapperB were higher expressions in U937R cells. These findings suggested resistance for I-BET151 in U937R cells might be related to the constitutive activation of NFkapperB signaling pathway via increased expressions of both BRD2 and BRD4. Targeting the NFkapperB signaling pathway could be effective therapeutic strategy to restore the sensitivity.

Development of the adenovirus vector systems
Because the adenovirus vector (AdV) is an attractive tool for gene expression and for the regulation of gene expression, it is applied to many areas of research. It is well known that the AdV is useful tool to transduce the purpose gene in hepatocytes. We develop a protocol for cure of hepatitis B virus (HBV) using AdV. In culture cells, the efficiency of HBV genome replication is poor. Therefore, we established the efficient genome replica-

tion of HBV applying AdVs (HBV103-AdV system). The HBV103-AdV-mediated HBV replication was easy and detected the replicated HBV genome in primary hepatocytes as well as in HepG2 cells. And also we were able to show that this system was useful for high-throughput screening of new anti-HBV drugs. Now we develop the completely HBV genome exclusion protocol using genome editing.

We also researched the efficient differentiation and enrichment methods to neural cells from induced pluripotent stem cells. We constructed 22 AdVs for this purpose. These AdV systems may contribute to the analysis of the cause of the neurologic disease.

Publications

Shinagawa S, Kobayashi N, Nagata T, Kusaka A, Yamada H, Kondo K, Nakayama K. DNA methylation in the NCAPH2/LMF2 promoter region is associated with hippocampal atrophy in Alzheimer's disease and amnesic mild cognitive impairment patients. *Neurosci Lett.* 2016 Aug 26; **629:** 33-7.

Suzuki A[1], Shibata N[1], Kasanuki K[1], Nagata T, Shinagawa S, Kobayashi N, Ohnuma T[1], Takeshita Y[1], Kawai E[1], Takayama T[1], Nishioka K[1], Motoi Y[1], Hattori N[1], Nakayama K, Yamada H, Arai H[1] ([1]Juntendo Univ). Genetic Association between Presenilin 2 Polymorphisms and Alzheimer's Disease and Dementia of Lewy Body Type in a Japanese Population. *Dement Geriatr Cogn Dis Extra.* 2016 Mar 16; **6:** 90-7.

Suzuki K[1], Yamamoto K[1], Arakawa Y, Yamada H, Aiba K, Kitagawa M[1] ([1]Juntendo Univ). Anti-myeloma activity of bromodomain inhibitors on the human myeloma cell line U266 by downregulation of MYCL. *Anticancer Drugs.* 2016 Sep; **27:** 756-65.

Takeshita Y[1], Shibata N[1], Kasanuki K[1], Nagata T, Shinagawa S, Kobayashi N, Ohnuma T[1], Suzuki A[1], Kawai E[1], Takayama T[1], Nishioka K[1], Motoi Y[1], Hattori N[1], Nakayama K, Yamada H, Arai H[1] ([1]Juntendo Univ). Genetic association between RAGE polymorphisms and Alzheimer's disease and Lewy body dementias in a Japanese cohort: a case-control study. *Int J Geriatr Psychiatr.* Epub 2016 Oct 4.

Kobayashi N, Shinagawa S, Nagata T, Shimada K, Shibata N[1], Ohnuma T[1], Kasanuki K[1], Arai H[1] ([1]Juntendo Univ), Yamada H, Nakayama K, Kondo K. Usefulness of DNA Methylation Levels in COASY and SPINT1 Gene Promoter Regions as Biomarkers in Diagnosis of Alzheimer's Disease and Amnestic Mild Cognitive Impairment. *PLoS One.* 2016 Dec 19; **11:** e0168816.

Suzuki M[1], Kondo S[1], Yamasaki M[2], Matsuda N[2], Nomoto A[2], Suzuki T[3], Saito I[1], Kanegae Y ([1]Tokyo Univ) ([2]Institute of Microbial Chemistry) ([3]Hamamatsu University School of Medicine). Efficient genome replication of hepatitis B virus using adenovirus vector: a compact pregenomic RNA-expression unit. *Sci Rep.* 2017; **7:** 41851.

Research Center for Medical Sciences
Core Research Facilities for Basic Science
(Division of Molecular Cell Biology)

Yoshinobu Manome, *Professor and Director*
Takeo Iwanoto, *Professor*
Keiichi Ikeda, *Assistant Professor*
Akihito Tsubota, *Professor*
Toshiaki Tachibana, *Professor*
Kouki Fujioka, *Assistant Professor*

General Summary

Core Research Facilities for Basic Science (Division of Molecular Cell Biology) was organized on April 1, 2014. The mission of the facilities is the facilitation of research in the university. Two systems are constituted for the use of our facilities.

1. Annual Registration System

This system is intended to supply research benches and other equipments to researchers of the university to perform experiments. Once registered, researchers can freely use the various devices in our institute. This system also provides technical advice and guidance on specific fine-morphological or biochemical approaches to a registrant's experiment, if necessary. In 2016, 160 researchers registered at our annual registration system and we provided 197 research supports for electron microscopy and 3 for laboratory experiments.

2. Research Support System

Advances in research technologies and equipment enable us to perform more precise and accurate observations of specimens in medical sciences. For researchers who cannot perform experiments owing to limits of time and funds, our staff can prepare samples for scanning electron microscopy and transmission electron microscopy, record images, or perform high-performance liquid chromatography and mass spectrometry. The service fee is minimal because services are limited to the university.

Research Activities

Change of MMPs expression of malignant brain tumor cells after exposure to anti-malignancy agent, temozolomide

Malignant brain tumors, especially malignant glioma and glioblastoma, are poor prognosis. Therefore, adjuvant therapies such as radiation and chemotherapy are required for the combination to surgical therapy. In this case, temozolomide is the first line drug used as an anti-neoplastic agent, but the influence on the invasivness of the tumor is major concern. While temozolomide suppresses proliferation of cell growth through methylation of DNA, it transiently activates transcription factors such as EGR-1 and NF-κB. Enzymes such as MMP9 chop off the basement membrane, and expression of the enzyme increases by transcriptional factors and enhance of the expression might affect invasion. In the study, gene transcription, protein amount, and activity of MMP2 and MMP9, were quantified after exposure to the therapeutic concentrations of temozolomide. As a result, the enzymatic activity of the MMP9 was not influenced by the agent. The therapeutic dose of

temozolomide was concluded less likely to have an effect on invasion via MMP activity. Also, feasibility of combination of temozolomide and MMP inhibitor was proposed.

Utilization of thermoresponsive magnetic nanoparticles with lower critical solutim temperature (LCST) in aqueous solution for detection of thyroid papillary carcinoma antigen
Thyroid carcinoma is one of the favorable prognostic malignancies in human body. For the reason, early diagnosis and treatment are important. While thyroid is easily reached by physical examination, the disease is generally diagnosed by ultrasound and fine needle aspiration. However, both methods have limitations. Previously, professor Takeyama Hiroshi, Department of Surgery in the university, established a monoclonal antibody for thyroid carcinoma and presented the effectiveness by immunostaining for clinical diagnosis. Whereas the result was obtained mainly by histological examination, the antigen was also present in blood. In current study, thermoresponsive magnetic nanoparticles with LCST in aqueous solution, was conjugated to the antibody for the application of the antibody for blood screening. Nanoparticles have larger surface and capacity for holding the larger amounts of antibody on the surface. The particle has thermoresponsive activity as well as magnetic properties, existence of antigen was detected by the method. Conjugation to the nanoparticle, the antibody may contribute to handy and more reliable screening of thyroid papillary carcinoma patients.

Human hepatocyte chimeric mice and hepatitis infection animal model
We have established human hepatocyte chimeric mice by an efficient method that we had developed and an animal model infected with hepatitis B or C virus by using the chimeric mice. Currently, we are intensely researching the efficacy of novel anti‑viral agents, the infection mechanism, and ultrastructural alterations of intrahepatocellular organelle after viral eradication.

Intrahepatic cellular localization of ATP7B
ATP7B protein, also known as Wilson disease protein, is a copper‑trans‑porting P‑type ATPase which is encoded by the *ATP7B* gene, locates in trans‑Golgi network of liver, and balances the copper level by excreting excess copper into bile and plasma. However, the exact localization of ATP7B in the hepatocyte is controversial and remains to be determined. We have been cooperating with the seminal research in The University of Barcelona (Spain).

SNPs, and RAVs in the treatment of chronic HCV infection
DAAs are the first‑line treatment for chronic HCV infection. We are investigating the association of SNPs of the genes with the blood drug concentration, treatment response, and DAA‑induced liver damage. RAVs are also being investigated in detail.

Comprehensive gene expression profiling analysis of microRNA/mRNA
We are profiling and analyzing the expression of microRNA/mRNA in the liver tissue of HBV‑infected human hepatocyte chimeric mice. We have found the novel interaction between microRNA and mRNA in HBV replication and lifecycle. We are also investigat-

ing the association between serum microRNA expression level and treatment outcome/prognosis in HCC patients who were treated with TACE/RFA.

Optimization of proteomics analysis by LC-MS bottom-up and top-down method
Currently, the analysis of the proteomics measured it by bottom up method or top-down method, and it was performed in combination with a powerful bioinformatics. Digested intact proteins in a restriction enzyme were measured by the bottom-up LC-MS/MS, and measurements ware provided peptide exact masses and fragment patterns. The protein database retrieval was performed based on these fragments pattern information and exact precursor molecular ion. These assigned proteins results were significantly useful for differential expression analyzes between a cancer tissue and the penumbra organization for a dynamic state profiling. To improve a detection limit, ionization efficiency was increased by using of matrix effected nanobooster and optimized LC separation condition. Now it was capable to identify 100 amol proteins routinely using nanoLC-MS/MS analysis. It was still difficult to obtain information about the PTM (phosphorylation, carbohydrate, drug conjugates) data using the conventional bottom-up method approach, but this information could be got by the LC-MS top-down method. In late years it was assumed that high molecular compounds detection including intact proteins ware difficult in spite of progress of the mass spectroscopy. Most of PTM studies were performed by immunoassays such as the Western blots and infusions MS method using highly pre-purified protein. Analysis of modification of carbohydrates and/or drug conjugates with intact protein were enabled at a routine level using optimized LC-ultra high resolution exact mass spectrometry device (Maxis3G) and Maximum Entropy Deconvoluted algorithm.

Isolation and characterization of embryonic ameloblast lineage cells derived from tooth buds of fetal miniature swine
Dental enamel formation, known as "amelogenesis," is initiated by cytodifferentiation of the ectodermally derived dental epithelium. Enamel cannot regenerate itself because once it is completely formed, ameloblasts are lost as the tooth erupts. Rodent teeth have been useful for studying the mechanisms of amelogenesis because ameloblast cell lines can be derived from the ever-growing incisors. However, higher mammals such as humans have no growing teeth, and cell lines derived from larger animals that are more similar to humans are required for higher fidelity studies. Here, we isolated embryonic enamel epithelium-derived epithelial cells from fetal swine. The explant culture of the developing deciduous molars that had been removed from the dental papilla-derived mesenchymal tissue and cells inside the tooth buds provided the epithelial cell population for the primary culture. To isolate the cell population, we performed a unique cell isolation technique called cell fishing. The isolated cells showed clear embryonic-stage ameloblast characteristics with appropriate gene/protein expressions of enamel matrix and proteinases, abundant glycogen pools, and secretory granular materials. They could be continuously subcultured several times and are presently being maintained. This cell population will facilitate the establishment of a stable cell line and allow us to characterize the definitive phenotype and functional behavior of porcine ameloblasts, which, in turn, promises to yield useful and practical findings that are more relevant than those provided by rodent

studies. Finally, analysis of in vitro enamel formation will be important for engineering "bio-enamel" as a new dental therapy to restore enamel defects.

Effects of urocortin III on insulin secretion from MIN6 mouse pancreatic β-cells in high glucose culture medium

We have been investigating the protective effects of urocortins, family peptides of CRH. We already reported that urocortin III stimulated insulin secretion from pancreatic β-cells in low to moderate high glucose (glucose concentration 1-4.5 g/L), but decreased in extremely high glucose condition (glucose concentration 9 g/L). Then, we also investigated the effects of urocortin III from the view point of histology. As the results, we confirmed that urocortin III increased dense-core granule at 1 g/L glucose, but decreased at 9 g/L glucose. These results indicate that urocortin III may differentially regulate insulin secretion as plasma glucose concentration in pancreatic β-cells.

Discrimination of volatile components using sensor description system

In this research, we aim to develop a system to objectively quantify fragrance by learning scent expressions using standards for sensor devices. Currently, GC and GC/MS are utilized for analysis of volatile components, and numerous components that are volatile components and biomarkers that characterize scents have been identified. On the other hand, when there are many types of volatile components, not only the component concentration but also the component ratio contained may be important for discrimination. Since the sensor can capture the characteristics of the whole scent, it can analyze from a different viewpoint from the current GC and GC/MS analyses, and it can analyze the flavor expression of food and the pattern discrimination of volatile biomarkers in the medical field. In order to achieve these objectives, we conducted training using flavor standards on sensor devices, and we developed an algorithm that expresses the characteristics of time-dependent changes during incubation of coffee which containing more than 600 chemical components as test samples. As a result, we found that fruity aroma increased with time. It is known that organic acids are produced by long-time keeping of coffee, and it is considered that these ingredients may have influences on the fruit scents. Thus, it was suggested that even with samples containing many kinds of volatile components, it is possible to express and discriminate change in scent easily and objectively by using our sensor description system. This research was supported by JSPS Grants-in-Aid for Science and Technology JP 16K12709.

Publications

Suzuki Y, Fujioka K, Ikeda K, Murayama Y, Manome Y. Temozolomide dose not influence the transcription or activity of matrix metalloproteinases 9 and 2 in glioma cell lines. *J Clin Neurosci.* 2017 Jul; **41:** 144-9. Epub 2017 Apr 10.

Hirabayashi T, Takahashi H[1], Watanabe M[1], Tachibana T ([1]Nippon Dental Univ). Establishment and characterization of a squamous cell carcinoma cell line, designated hZK-1, derived from a metastatic limph node tumor of the tongue. *Human Cell.* Epub 2017 Mar 4.

Kondo C[1], Atsukawa M[1], Tsubota A, Shimada N[2], Abe H[3], Asano T[4], Yoshizawa K[5], Okubo T[1], Chuganji Y[4], Aizawa Y, Tanaka Y[6], Iwakiri K[1] ([1]Nippon Medical School, [2]Ootakanomori Hosp, [3]Shinmatsudo Central Hosp, [4]Tokyo Metropolitan Bokutoh Hosp, [5]Machida Municipal Hosp, [6]Nagoya City Univ). Daclatasvir and

asunaprevir for genotype 1b chronic hepatitis C patients with chronic kidney disease. *Hepatol Res.* 2017 Oct; **47:** 1165–73. Epub 2017 Apr 12.

Inoue T*[1]*, Hmwe SS*[2]*, Shimada N*[3]*, Kato K*[4]*, Ide T*[5]*, Torimura T*[5]*, Kumada T*[6]*, Toyoda H*[6]*, Tsubota A, Takaguchi K*[7]*, Wakita T*[2]*, Tanaka Y*[1] *([1]Nagoya City Univ, National Institute of Infectious Diseases, [3]Ootakanomori Hosp, [4]Shin-Matsudo Central Hosp, [5]Kurume Univ School of Medicine, [6]Ogaki Municipal Hosp, [7]Kagawa Prefectural Central Hosp). Clinical Significance of Two Real-Time PCR Assays for Chronic Hepatitis C Patients Receiving Protease Inhibitor-Based Therapy. *PLoS One.* 2017 Jan 24; **12:** e0170667.

Kato K*[1]*, Abe H*[1]*, Ika M*[1]*, Yonezawa T*[1]*, Sato Y*[1]*, Hanawa N*[1]*, Shimizu S*[1]*, Endo S*[1]*, Matsuo R*[1]*, Tsubota A ([1]Shinmatsudo Central Hosp). C-Arm Cone Beam Computed Tomography Guidance for Radiofrequency Ablation in Hepatocellular Carcinoma. *Oncology.* 2017 Jan 5; **92:** 142–52.

Furihata T*[1]*, Morio H*[1]*, Zhu M*[1]*, Suzuki Y*[1]*, Ide H*[1]*, Tsubota A, Fu Z*[1]*, Anzai N*[1]*, Chiba K*[1] *([1]Chiba Univ). Human organic anion transporter 2 is an entecavir, but not tenofovir, transporter. *Drug Metab Pharmacokinet.* 2017 Feb; **32:** 116–9.

Fujioka K, Iwamoto T, Shima H*[1]*, Tomaru K, Saito H, Ohtsuka M*[1]*, Yoshidome A*[1]*, Kawamura Y*[1]*, Manome Y ([1]Sharp Coorp). The powdering process with a set of ceramic mills for green tea promoted catechin extraction and ROS inhibition effect. *Molecules.* 2016 Apr 11; **21:** 474.

Suzuki Y, Watanabe M, Murayama Y, Karagiozov K, Manome Y, Ohashi H. Usefulness of the behavior of fibroblast attachment to coils in thermoreversible gelation polymer for aneurysmal coil. *Translational Medicine (Sunnyvale).* 2016; **6:** 1000167.

Altinel K*[1]*, Hashimoto K*[1]*, Wei Y*[2]*, Neuveut C*[2]*, Gupta I*[1]*, Suzuki AM*[1]*, Dos Santos A*[3]*, Moreau P*[2]*, Xia T*[2]*, Kojima S*[1]*, Kato S*[1]*, Takikawa Y*[4]*, Hidaka I*[5]*, Shimizu M*[6]*, Matsuura T, Tsubota A, Ikeda H*[7]*, Nagoshi S*[8]*, Suzuki H*[1]*, Michel ML*[2]*, Samuel D*[3]*, Buendia MA*[3]*, Faivre J*[3]*, Carninci P*[1] *([1]RIKEN Center, [2]Institut Pasteur, [3]Paul-Brousse Hosp, [4]Iwate Medical Univ, [5]Yamaguchi Univ, [6]Gifu Univ, [7]Tokyo Univ, [8]Saitama Medical Univ). Single-Nucleotide Resolution Mapping of Hepatitis B Virus Promoters in Infected Human Livers and Hepatocellular Carcinoma. *J Virol.* 2016 Nov 14; **90:** 10811–22.

Kinoshita C, Nagano T, Seki N, Tomita Y, Sugita T, Aida Y, Itagaki M, Satoh K, Sutoh S, Abe H, Tsubota A, Aizawa Y. Hepatitis C virus G1b infection decreases the number of small low-density lipoprotein particles. *World J Gastroenterol.* 2016 Aug 7; **22:** 6716–25.

Okubo T*[1]*, Atsukawa M*[1]*, Tsubota A, Shimada N*[2]*, Abe H*[3]*, Yoshizawa K*[4]*, Arai T*[1]*, Nakagawa A*[1]*, Itokawa N*[1]*, Kondo C*[1]*, Aizawa Y, Iwakiri K*[1] *([1]Nippon Medical Sch, [2]Ootakanomori Hosp, [3]Shinmatsudo Central Hosp, [4]Machida Municipal Hosp). Association between vitamin D deficiency and pre-existing resistance-associated hepatitis C virus NS5A variants. *Hepatol Res.* 2016 Aug 2.

Shigetani Y, Wakamatsu Y*[1]*, Tachibana T, Okabe M ([1]Tohoku Univ). Conversion of neural plate explants to pre-placodal ectoderm-like tissue in vitro. *Biochem Biophys Res Com.* 2016 June 28; **477:** 807–13.

Nakagawa A*[1]*, Atsukawa M*[1]*, Tsubota A, Kondo C*[1]*, Okubo T*[1]*, Arai T*[1]*, Itokawa N*[1]*, Narahara Y*[1]*, Iwakiri K*[1] *([1]Nippon Medical Sch). Usefulness of portal vein pressure for predicting the effects of tolvaptan in cirrhotic patients. *World J Gastroenterol.* 2016 Jun7; **22:** 5104–13.

Iio E, Shimada N, Abe H, Atsukawa M, Yoshizawa K, Takaguchi K, Eguchi Y, Nomura H, Kuramitsu T, Kang JH, Matsui T, Hirashima N, Tsubota A, Kusakabe A, Hasegawa I, Miyaki T, Shinkai N, Fujiwara K, Nojiri S, Tanaka Y. Efficacy of daclatasvir/asunaprevir according to resistance-associated variants in chronic hepatitis C with genotype 1. *J Gastroenterol.* 2017 Jan; **52:** 94–103.

Itokawa N*[1]*, Atsukawa M*[1]*, Tsubota A, Okubo T*[1]*, Arai T*[1]*, Nakagawa A*[1]*, Kondo C*[1]*, Iwakiri K*[1] *([1]Nippon Medical School). Effects of sorafenib combined with low-dose interferon therapy for advanced hepatocellular carcinoma: a pilot study. *Int J Clin Oncol.* 2016 Aug; **21:** 676–83.

Research Center for Medical Sciences Laboratory Animal Facilities

Hirotaka Kanuka, *Professor and Director* Tatsuya Sakurai, *Assistant Professor*

General Summary

The purpose of the Laboratory Animal Facilities is to support *in-vivo* research and to contribute to the development of basic and clinical medicine. In 2016, 698 researchers were registered as users of the Laboratory Animal Facilities. We undertake breeding of experimental animals and provide technical guidance to researchers in animal experimentation. In addition, we performed the following studies to develop basic medical sciences, including laboratory animal science.

Research Activities

Studies of parasite-vector and parasite-host interactions of African trypanosomes

African trypanosomiasis is a deadly protozoan disease of humans and animals. The disease is caused by African trypanosomes, which are transmitted by tsetse flies (*Glossina* spp.). To adjust to the mammalian host and insect vector environments, the parasite has a complicated lifecycle involving developmental stages. The bloodstream forms are parasitized in the bloodstream of the vertebrate hosts. During blood feeding of tsetse, bloodstream forms are taken up and differentiate to procyclic forms which lack host-infectivity in the midgut. Subsequently, procyclic forms migrate to tsetse salivary gland or proboscis where they differentiate to the epimastigote forms. The epimastigote forms strongly adhere to tsetse tissue, proliferate, and differentiate into animal infective metacyclic forms. The differentiation (from the epimastigote form to metacyclic form) is called metacyclogenesis, which is indispensable for the parasite to be cyclically transmitted. The cell adhesion of the epimastigote forms are known to be essential for the metacyclogenesis of *Trypanosoma congolense*, the cause of animal African trypanosomiasis. By using *T. congolense*, we are trying to elucidate the molecular mechanisms underlying metacyclogenesis through transcriptome analyses on the epimastigote forms whose cell adhesion and subsequent metacyclogenesis are inhibited.

Development of a novel immunological method of fecal occult blood testing for dogs and fecal occult blood trend in digestive diseases

With advances in veterinary medicine, the lives of companion animals, such as dogs and cats, have been extended. On the other hand, neoplastic diseases have also been increasing, and the development of screening methods has become an urgent task. The fecal occult blood test (FOBT) is a method for detecting in feces a small amount of blood that is undetectable with the naked eye or under a microscope. The FOBT was originally developed as a screening test for alimentary canal tumors in human patients. However, the FOBT remains rarely used in veterinary medicine. In addition, little is known about

its clinical significance, because the chemical FOBT is based on the peroxidase activity of hemoglobin. Thus, this chemical test had low sensitivity and specificity and was not suitable for dogs, which live in various environments today. We developed a novel FOBT test using laser nephelometric immunoassay for dogs and investigated its performance. We demonstrated that our immunological FOBT method is independent of a dog's diet. We also demonstrated that infection with a specific type of gastrointestinal parasite causes a significant increase of FOBT values in dogs and that this increase was significantly decreased with anthelmintic treatment. We are now evaluating cases of gastrointestinal cancer in dogs over time and investigating the diagnostic value of our FOBT method.

Preventing malaria by adjusting amino-acid intake

Preventive and therapeutic methods against malaria, a major parasitic disease, need to be established because of the emergences of multiple drug-resistant *Plasmodium* strains. Malaria is caused by *Plasmodium* parasite, and this parasite is incapable of most types of amino acid biosynthesis, depending on a part of the amino acid source on free amino acids in plasma. Thus, we are searching for a method of malaria control based on nutritional knowledge by performing the global analysis of amino acid composition in plasma (plasma aminogram analysis). With an *in vivo* murine model, we have shown that the treatment of mice with isoleucine deficient diet (Ile-def diet) significantly inhibited parasitemia. A combination studies with Ile-def diet and artesunate, the first-line drug against malaria, indicated that this food has synergistic effect with antimalarial agents. Furthermore, Ile-def diet treatment prolonged the survival of the mice that is experimental model of cerebral malaria. Currently, using an *in vivo* murine model, we are studying the effect of Ile-def diet on liver-stage parasite development.

Research Center for Medical Sciences
Radioisotope Research Facilities

Kunihiko Fukuda, *Professor and Director*
Tadashi Asakura, *Professor*
Haruka Minowa, *Assistant Professor*

General Summary

The Radioisotope Research Facilities were established to support medical and biological research using radioisotopes. The Facilities also accept the research using non-radioactive isotopes. We have supported researchers by suggesting methods and practical techniques for experiments. Lectures and training courses are held for researchers and for medical students and graduate students. In 2016, 35 researchers from 11 departments and 8 students of 2 curriculums used the laboratory of this facility. Major nuclides used for experiments were ^{32}P, ^{51}Cr, ^{125}I, ^{14}C, and ^{3}H.

The Fukushima Dai-ichi Nuclear Power Plant was damaged by the Tohoku-Pacific Ocean Earthquake on March 11, 2011. Large amounts of fallout were released into the environment by the accident. We focus on the study of the behavior and distribution of the radioactive materials in the environment. Education related to radiation is also an interest.

Proteasome inhibitors are drugs with highly anticipated efficacy as clinical anticancer drugs. One such inhibitor, PS-341, is already being used to treat multiple myeloma. However, little data is available on the clinical use of proteasome inhibitors as anticancer drugs. If a proteasome inhibitor has systemic side effects or if cancer cells have become resistant and reappear after inadequate or incomplete cancer therapy, this type of agent must be administered with extreme care. To evaluate the generation of inhibitor-resistant cells and their specific properties, a strategy for second-line chemotherapy must be developed.

Research Activities

Expression of ZEB1 by repression of microRNA-200 (miR200) in proteasome inhibitor-resistant cells may be suppressed E-cadherin

Endometrial carcinoma Ishikawa which acquired resistance for Epoxomicine (EXM), a proteasome inhibitor caused epithelial-mesenchymal transition (EMT) with the onset of E-cadherin disappearance. I made clear that transcription repressor ZEB1 regulated expression of E-cadherin.

We examined expression of E-cadherin in the microRNA (miR) expression recently because regulation of various kinds of genetic expression by miR was reported. We measured expression of miR in Ishikawa and EXM-resistant Ishikawa (Ish/EXM) as follows: miR9, miR10a, miR10b, miR21, miR23a, miR23b, miR34a, miR141, miR150, miR192, miR200a, miR200b, miR200c, miR205, miR206, miR215, miR217, miR221, miR298, miR374b, miR382, miR429, miR508-3p, miR539. As a result, miR10a and miR10b expressed in Ish/EXM, and miR141, miR200a, miR200b and miR200c suppressed in Ish/

EXM. Because homogeny of the base sequence was high, miR141, miR200a, miR200b and miR200c were called miR200 family, and the regulation of EMT by miR200 family was reported, we examined expression of transcription inhibitor ZEB1.
The miR200 family was highly expressed in Ishikawa where E-cadherin expressed here, and expression of ZEB1 disappeared, and expression of miR200 family disappeared in Ish/EXM where expression of E-cadherin disappeared, and ZEB1 expressed. In addition, because the expression of miR200 family which disappeared was not restored even if I knocked down ZEB1 of Ish/EXM, miR200 family was located in the upstream of ZEB1. Therefore, we regulated expression of miR200 family in Ishikawa and Ish/EXM by transfection of anti-miRNA and pre-miRNA. When we transfected anti-miR200 and knocked down miR200 of Ishikawa which expressed of miR200, ZEB1 appeared, and expression of E-cadherin was repressed with expression of ZEB1. When we transfected pre-miR200 into Ish/EXM which disappeared of miR200 and produced miR200, ZEB1 disappeared, and expression of E-cadherin was restored. These turned out similar at a gene level and protein level, and it was suggested that miR200 family was located in the upstream of ZEB1.

Analysis of resistance mechanisms in radiation-resistant organisms
Tardigrades, which are called water bears, can tolerate extreme environments, including ionizing radiation and dryness. The sludge water bear *Isohypsibius* were isolated from the activated sludge in Mikawajima Water Reclamation Center, and the terrestrial water bear *Milnesium tardigradum* were isolated from moss collected at Minato Ward in Tokyo. To clarify the radiation-resistant mechanism, tardigrades were irradiated with X-ray at 50 to 300 Gy, and DNA damage was analyzed with the comet assay method.

Measuring and tracing of radioactive fallout in the environment
The distribution and behavior of radioactive fallout released into the environment by the accident of the Fukushima Daiichi Nuclear Power Plant in March 2011 have been investigated. Because contaminated water had been leaked into the ocean by accident, we recently examined a safe, simple and rapid method of analyzing radioactive strontium in seawater. Radioactive strontium was separated by a column of cation exchange resin (Dowex 50WX8, Dow Chemical Company, Midland, MI, USA) and was measured using newly developed plastic scintillator bottle with liquid scintillation system (LSC-LB7, Hitachi Ltd.). With this method, the chemical separation of 10 hours (total 2 days) could be evaluated and compared with 2 weeks with a conventional technique. The detection limit in this procedure from 200 mL of seawater was 0.1 Bq/L. This method might be able to be used to survey contaminated seawater.

Study of radon
Radon, which is a gaseous radioactive element, dissolves in groundwater and hot springs and then reaches the surface of the ground. The radon contamination in groundwater reflects the underground structure. We measured the radon concentrations of 57 springs that has been designated by the Tokyo Metropolitan Government Bureau of Environment and discussed the geological features of Tokyo.

Publications

Yokoyama T, Misawa K, Okano O, Minowa H, Fukuoka T. Photostimulated luminescence applicable to pre-screening of potassium-rich phases in chondritic breccias. *J Radioanal Nucl Chem.* 2016; **310:** 81-9.

Research Center for Medical Sciences GMP Production Facilities for Cell Therapy and Gene Therapy

Sadamu Homma, *Professor and Director* Tomoko Ohmae, *Assistant Professor*

General Summary

This facility was established for clinical studies based on cell therapy, gene therapy and regenerative medicine. Cell products are generated here on the standard of Good Manufacturing Practice (GMP) for safe administration to the patients in clinical studies. Specified regulation and education have been performed strictly for maintenance of the GMP standard in this facility.

Research Activities

In 2016, a new law, regenerative medicine safety assurance Act, is enforced and all the activities concerning cell therapy including dendritic cell therapy against malignancies must be under the regulation of this law. To continue dendritic cell therapy against glioblastoma multiforme (GBM), that has been performed for more than 10 years in Jikei University Hospital, the procedures for compliance with the new law was required. First, the certified regenerative medical commission (class 3) was established in Jikei University to review the dendritic cell therapy against GBM or other cell therapies. Second, the GMP production facility for cell therapy and gene therapy in Jikei University was inspected by PMDA to be approved as a cell processing center under the new law. Finally, this facility was approved as a certified cell processing center and the dendritic cell therapy against GBM was accepted by Kanto/Shin-etsu division of public welfare in the ministry of health, labour and welfare. Although generation of dendritic cell vaccine using this facility had been stopped during this legal procedure, it started again upon the acceptance. These legal procedures have contributed to the future performance of cell therapy and regenerative medicine under the compliance with the new law in Jikei University.

Research Center for Medical Sciences
Institute for High Dimensional Medical Imaging

Naoki Suzuki, *Professor* Asaki Hattori, *Associate Professor*

General Summary

The goal of our research is to develop new imaging systems that can be applied to clinical medicine now and in the future. High-dimensional, i.e., 3-dimensional (3D) and 4-dimensional (4D), imaging techniques have enabled noninvasive, realistic, uninhibited, and accurate observations of human spatial structures and their dynamics. The availability of real-time imaging with high-performance computers and medical virtual reality systems has expanded the possibilities for diagnosis, treatment, surgery, and medical education. The Institute for High Dimensional Medical Imaging has, therefore, established a system that facilitates cooperative research and development with international researchers and organizations.

Research Activities

Clinical application of high-definition, real-time medical imaging

We are performing research on the development of medical high-definition imaging technology and its clinical application using functional and morphological data obtained with X-ray computed tomography (CT) and magnetic resonance imaging.

We are developing a 4D motion system for analyzing human activities, such as the motions of the whole body. The system is driven by motion data obtained from anatomical and skeletal muscle models reconstructed from X-ray CT data sets. In this year, the evaluation of the deformation of the skeletal muscle model using MRI was performed together with the Department of Radiation in the development of a four-dimensional human body model capable of deforming soft tissues (skin, abdominal organs, skeletal muscles, vasculature, etc.) in whole body movements. It is difficult for MRI currently used in clinical practice to measure regions having certain capacity as volume data like MDCT at high speed. Therefore, we developed equipment, which the participant can repeat a certain action in a stable manner with the similar load applied as walking in the gantry. We also developed a device capable of synchronizing the motion of participants and MRI imaging, and conducted a clinical trial by examining a sequence capable of photographing in a large spatial resolution of a wide region of certain extent. We also continue to develop a system that predicts and visualizes future growth of children using multiple X-ray CT data measured in the past.

From this year, we began developing a three-dimensional shape evaluation method of outside nose cartilage with the Department of Plastic and Reconstructive Surgery. For outside nasal cartilage, which is said to be difficult to detect by normal image examination, we examined imaging technique using X-ray CT and MRI with the Department of Radiation. This research aims not only to evaluate the cartilage shape but also to develop

a system that performs surgical planning and surgical simulation based on the obtained results.

Development of endoscopic surgical robot system
We are developing an endoscopic surgical robot system that can be used to perform natural orifice transluminal endoscopic surgery (NOTES). Robotic instruments enter the abdominal cavity orally and are used to perform surgery on the abdominal organs. Following our research from the previous year, this year, we are refining the driving mechanism of the over tube flexing mechanism to maintain the posture in the abdomen of the robot.
In addition, in development of a multi-view camera system suitable for endoscopic surgery and robot surgery, we were able to acquire patents based on research results.

Development of a surgical simulator for various surgical techniques
We are developing a simulator that can deal with various surgeries, such as laparotomy and endoscopic surgery, using preoperative X-ray CT data of a patient.
This year, we developed a system that reflects the results of preoperative surgical simulation in intraoperative navigation and examined its usefulness in clinical trials of navigation surgery as described later. Moreover, in the development we are conducting from last year, of a 4-dimensional image display system to real space where the surgeon can intuitively grasp the anatomy structure, the research proposal planned based on this basic experiment was chosen for JSPS Grant-In-Aid for Scientific Research (A).

Development of an image-guided surgery system
We are developing a system that can display blood vessels and tumors at the back of the surgical field in the form of 3D geometric models in multiple layers on the surgical field screen. Such improvements will make the navigation system more intuitive. This year the Department of Surgery and the Department of Otorhinolaryngology again jointly performed navigation surgery in the high-tech navigation operating room of Daisan Hospital as a semiroutine procedure. Especially, in this year, research results on intraoperative navigation system using tablet PC which we are developing with the Department of Surgery, won a prize at an international conference.
In addition, from this year, we started basic experiments for development of intraoperative navigation system with the Department of Obstetrics and Gynecology. Because the gynecologic and gynecological field is an area in which intraoperative navigation has been rarely performed, we examined the selection of parts and blood vessels necessary for navigation and the presentation method by basic experiments.

Application of high-definition medical image analysis to forensic medicine
By applying technology that we have developed for analyzing high-definition medical images, we are analyzing X-ray CT data sets of crime victims with the aim of developing new methods for future criminal investigations and for establishing new methods for creating court documents. As we did last year, this year we carried out 3D analyses of the position, depth, and angle of the attempted-murder victim's injuries using the victim's

X-ray CT data set.

Publications

Kimura T, Kubota M, Taguchi T, Suzuki N, Hattori A, Marumo K. Evaluation of first-ray mobility in patients with hallux valgus using weight-bearing CT and a 3D analysis system: A comparison with normal feet. *J Bone Joint Surg.* 2017 Feb; **99:** 247-55.

Research Center for Medical Sciences
Institute of Clinical Medicine and Research

Toya Ohashi, *Professor and Director* Takashi Sasaki, *Professor*

General Summary

In addition to performing our own research activities, in 2016 we continued to engage in an educational laboratory course program with the assignment of students of the third-year grade from the School of Medicine in 2016. We also fulfilled research support duties for registered researchers from the University Hospital at Kashiwa campus (Departments of Gastroenterology; Laboratory Medicine; Diabetes, Metabolism and Endocrinology; and General Internal Medicine) so that doctor physician-researchers could work freely. Their research work has been progressed efficiently.

Research Activities

Mechanism of islet injury and beta cell regeneration in diabetes mellitus

Pancreatic islet structure includes peripheral nerve fibers that are non-endocrine cells, capillaries, and ectoderm oriented, such as neural crest-derived peri-islet Schwann cells also make up the islet structure. It has been estimated that the function of the Schwann cell might have the same functions as the same as the function of allogeneic astrocytes and Schwann cells in the other tissues of the nervous system. These functions might include the supplementation of nutrients to the blood vessels and the endocrine cells and shielding cells from exogenous stress, but the functions remain unclear.

Research to elucidate the structure-function relationship of the islet compartment structure and molecules for the cell to cell communication should be helpful to understand the origins of pancreatic islet failure in diabetes mellitus. We have already started a study of "beta cell protection from metabolic stress" through the islet architecture. Experiments in 2016 showed that, in the co-culture conditions of MIN6, a murine established beta cell line, and IMS32, a murine established Schwann cell line, significantly higher in GSIS (glucose-stimulated insulin secretion) or insulin secretory capacity than in MIN6 of a single culture system. When expression of mRNA for the molecule that performs intercellular communication, gapjunction, was knocked-out with RNA interference technology, GSIS was lowered than in the control. This phenomenon was considered as a protective effect from the Schwann cells via intercellular communication.

Search for novel biomarker in skin gas with gas chromatography

Continuing from the previous fiscal year, we searched for a methodology for of detection with skin-derived gas-by-gas chromatography to find novel biomarkers for metabolic or physical stress including systemic inflammation.

Study of the change of the body components during treatment of diabetes mellitus by sodium-dependent glucose co-transporter SGLT2 inhibitor

In the treatment of type 2 diabetes by with dietary restrictions and medication, changes of body composition change associated, in particular, with the possibility of muscle loss and body fat increase, have become a problem. Sodium-dependent glucose co-transporter (SGLT2) inhibitor is known to cause body weight loss accompanied by body fat reduction, but details of body-composition change are not known. Furthermore, concerns have been raised about a possible worsening prognosis because of a decrease in skeletal muscle mass (sarcopenia). To clarify these issues, we have performed a multicenter, open-labeled follow-up clinical study with of an SGLT2 inhibitor on in Japanese patients with type 2 diabetes. So far 11 medical facilities are involved in this prospective study. In the analysis, body fat mass was found to have continued to decrease efficiently for up as long to as 52 weeks with dual-energy X-ray absorptiometry, DXA. Skeletal muscle mass decreased slightly during the initial 12 weeks yet showed no further decrease after 24 weeks.

Publications

Sakai S*[1]*, Kaku K*[2]*, Seino Y*[3]*, Inagaki N*[4]*, Haneda M*[5]*, Sasaki T, Fukatsu A*[6]*, Kakiuchi H*[1]*, Samukawa Y*[1] *([1]Taisho Pharmaceutical Co., Ltd., [2]Kawasaki Medical Sch, [3]Kansai Electric Power Hosp, [4]Kyoto Univ, [5]Asahikawa Medical Univ, [6]Yachiyo Hosp). Efficacy and Safety of the SGLT2 Inhibitor Luseogliflozin in Japanese Patients with Type 2 Diabetes Mellitus Stratified According to Baseline Body Mass Index: Pooled Analysis of Data From 52-Week Phase III Trials. *Clin Ther.* 2016; **38:** 843-62.

Yanai H*[1]*, Hirowatari Y*[2]*, Ito K*[3]*, Kurosawa H*[4]*, Tada N, Yoshida H ([1]Natl Center for Global Health and Medicine Kohnodai Hosp, [2]Saitama Prefectural Univ, [3]Yaesu Sakura Dori Clinic, [4]Inzai General Hosp). Understanding of Diabetic Dyslipidemia by Using the Anion-Exchange High Performance Liquid Chromatography Data. *J Clin Med Res.* 2016; **8:** 424-6.

Shibahara-Sone H*[1]*, Gomi A*[1]*, Iino T*[1]*, Kano M*[1]*, Nonaka C*[1]*, Watanabe O*[1]*, Miyazaki K*[1]*, Ohkusa T ([1]Yakult Central Institute). Living cells of probiotic Bifidobacterium bifidum YIT 10347 detected on gastric mucosa in humans. *Benef Microbes.* 2016; **7:** 319-26.

Akasaki Y, Kikuchi T, Homma S, Koido S, Ohkusa T, Tasaki T, Hayashi K, Komita H, Watanabe N, Suzuki Y, Yamamoto Y, Mori R, Arai T, Tanaka T, Joki T, Yanagisawa T, Murayama Y. Phase I/II trial of combination of temozolomide chemotherapy and immunotherapy with fusions of dendritic and glioma cells in patients with glioblastoma. *Cancer Immunol Immunother.* 2016; **65:** 1499-509.

Manita D*[1]*, Yoshida H, Hirowatari Y*[2] *([1]Tosoh Corporation, [2]Saitama Prefectural Univ). Cholesterol Levels of Six Fractionated Serum Lipoproteins and its Relevance to Coronary Heart Disease Risk Scores. *J Atheroscler Thromb.* 2017 Sep; **24:** 928-39. Epub 2016 Dec 26.

Reviews and Books

Kajihara M, Takakura K, Kanai T, Ito Z, Matsumoto Y, Shimodaira S*[1] *([1]Shinshu Univ), Okamoto M*[2] *([2]Kitasato Univ), Ohkusa T, Koido S. Advances in inducing adaptive immunity using cell-based cancer vaccines: Clinical applications in pancreatic cancer. *World J Gastroenterol.* 2016; **22:** 4446-58.

Kajihara M, Takakura K, Kanai T, Ito Z, Saito K, Takami S, Shimodaira S*[1] *([1]Shinshu Univ), Okamoto M*[2] *([2]Kitasato Univ), Ohkusa T, Koido S. Dendritic cell-based cancer immunotherapy forcolorectal cancer. *World J Gastroenterol.* 2016; **22:** 4275-86.

Koido S, Okamoto M*[1]*, Shimodaira S*[2]*, Sugiyama H*[3] *([1]Kitasato Univ, [2]Shinshu Univ, [3]Osaka Univ). Wilms' tumor 1 (WT1)-targeted cancer vaccines to extend survival for patients with pancreatic cancer. *Immunotherapy.* 2016; **8:** 1309-20.

Koido S. Dendritic-Tumor Fusion Cell-Based Cancer Vaccines. *Int J Mol Sci.* 2016; **17:** 828.

de Carvalho LS*[1]*, Yoshida H ([1]State Univ Campinas, Brazil). Monthly PCSK9 inhibitors: The CHOICE for prolonged duration of effect. *Atherosclerosis.* 2016; **254:** 300-2.

Centers of Advanced Medicine
Center for Neuroscience of Pain

Fusao Kato, *Professor and Director*

General Summary

The Jikei Center for Neuroscience of Pain (JCNP) was established in April 2014 as the first member of the Core Centers for Advanced Medicine of The Jikei University as a stronghold to advance the research, clinical and biomedical, in the Jikei University under the support of the Ministry of Education, Culture, Sports, Science and Technology–Supported Program for the Strategic Research Foundation at Private Universities (S1311009; FY2013–2017).

Pain is one of the most serious concerns in medicine. Besides being a beneficial physiological alarm for on-going harmful events, such as the injury and inflammation, pain is not only a simple sensation but it is inevitably suffering, being accompanied by strong negative emotions. This latter characteristic also helps patients remember the potentially harmful situations to change their future behaviors. However, such emotional aspect of pain also results in decreased quality of life. It prevents concentration and rest and leads the patients to various mental disorders including depression and anxiety. These changes often lead to various psychosomatic complications. Thus, identifying the cerebral mechanism underlying the emotional aspect of pain is an urgent issue to alleviate, control, and mitigate the patient's suffering from a large variety of pain symptoms described in various parts of the body, such as the head, back, viscera, and even absent limbs (e.g., "phantom limb pain" syndrome). Recent advances in pain science have identified the networks of the brain as the nuclear mechanism responsible for such clinically "undesired" pain. In particular, chronic pain, which numerous patients claim (>15% of the population in major countries), is now thought to be established through changes in the widely distributed neural networks underlying the sensory, cognitive, and affective dimensions of pain. The JCNP has been successful in integrating the activities of the diverse research teams in The Jikei University and other institutions to establish a basis for advances to be made in understanding, evaluating, and mitigating unnecessary pain.

Research Activities

The JCNP is composed of 3 research cores

1. Core for the brain mechanism of pain (core leader, Fusao Kato)

This core studies the brain plasticity mechanism underlying the establishment of chronic pain using neurophysiological, neuroanatomical, and neuropharmacological approaches with techniques from molecular biology, behavioral sciences, to optogenetics/pharmacogenetics. In addition, this core utilizes ultrahigh magnetic field magnetic resonance imaging for small animals at The Jikei University to visualize brain activity during the chroni-

fication process of pain (in which pain changes from episodic to chronic) and to evaluate the effects of various therapeutic interventions, such as the transcranial magnetic stimulation.

2. Core for the specific disease-associated pain (core leader, Toya Ohashi)

This core aims to identify mechanisms underlying aberrant specific pain accompanying specific types of diseases, such as Fabry disease, syringomyelia, poststroke pain, postherpes pain, fibromyalgia, and painful diabetic neuropathy. Taking advantage of The Jikei University Hospital, which is visited by many patients with these diseases, this core will use various approaches, including animal models of disease, primary cultured cells, and induced pluripotent stem cells derived from patients, and attempt to translate the findings in the animals to clinical applications.

3. Core for the pain in human patients (core leader, Shoichi Uezono)

This core deals with the pain of multiple etiologies frequently observed in patients. Such pain includes postoperative pain, cancer pain, and neuropathic pain, most of which are resistant to therapy and have unidentified mechanisms. Collaborations between divisions for biomedical sciences in other cores and clinical departments, such as anesthesiology (including the pain clinic), rehabilitation medicine, orthopedic surgery, neurology, and neurosurgery, are promoted in this core. The detailed clinical analyses of the sensory, cognitive, and affective dimensions of pain in relation to other clinical observations in each patient will be used to develop and examine novel strategies against therapy-resistant complications of chronic pain.

Close mutual interactions between these cores are promoted with the strong leadership of the directors and the Department of Neuroscience, where the head quarter of the JCNP is located. In addition, advanced experimental systems for pain evaluation and brain activity measurement are installed in the Department of Neuroscience, which are now frequently used by many researchers belonging to the JCNP.

Centers of Advanced Medicine
Center for Medical Entomology

Hirotaka Kanuka, *Professor*
Kenji Ishiwata, *Associate Professor*
Tatsuya Sakurai, *Assistant Professor*

General Summary

Arthropod vectors are organisms that play a role in the transmission of a pathogen between humans or from animals to humans. Vectors tend to be blood-sucking insects that ingest the disease-causing organism with the blood from an infected host and then inject it into a new host at the time of their next blood-meal. New strategy to control the vector should absolutely be developed and involved in integrated vector management (IVM), because it is one of the most effective means of dealing with the problem while waiting for a vaccine or another effective dengue control strategy. In this center, based on collaboration between our center and institutions in endemic countries such as Burkina Faso, Nigeria, and Taiwan, entomological studies promoting multilateral approaches have been performed to gather fine knowledge of diagnosis, ethology, immunity, and epidemiology of vector species on effective vector control.

Research Activities

Symbiotic bacteria Wolbachia *manipulate host germline stem cells by targeting host RNAs*

Wolbachia are the most abundant intracellular bacteria, infecting >65% insect species. The global *Wolbachia* pandemic is maintained by their ability to manipulate host biology in diverse ways such as feminization, parthenogenesis, male killing, cytoplasmic incompatibility and viral protection. *Wolbachia* have received attention for use in controlling insect pests and disease vectors by applying their biology. However, contrary to the advances in their practical use, the mechanisms how *Wolbachia* manipulate host cellular functions are largely unknown. To elucidate the interactions between *Wolbachia* and hosts at the molecular level, we aimed to identify *Wolbachia* factors controlling host cells and reveal their functions. To achieve this goal, we adopted a novel strategy employing a heterologous expression system using *Drosophila* genetics. As a result, we identified one *Wolbachia* gene named *TomO* (*TOxic Manipulator of Oogenesis*) which recapitulated some of the *Wolbachia*'s effects on *Sex-lethal* (*Sxl*) mutants of *D. melanogaster*. The Sxl is the master regulator of the sex determination system in *D. melanogaster* females. In *Sxl* mutant females, the germline stem cells (GSCs) were masculinized and lost, otherwise *Wolbachia* infection rescued the aberration. The heterologous expression of TomO in *Drosophila* GSCs also prevented the loss of the mutants GSCs. We revealed that TomO targets host *nanos* mRNAs, which are responsible for the maintenance of GSCs, and enhances their translation. Considering that the regulation of specific RNAs could mediate the diverse *Wolbachia*-induced phenomena, it will be of great interest to examine the

involvement of TomO.

Genetic dissection of mosquitoes adaptation to different hosts
Zoophilic mosquitoes bite animals and maintain infectious diseases in the sylvatic cycle. However, humans are occasionally bitten by zoophilic mosquitoes and receive causal agents of infectious diseases from animals. This shift in biting is responsible for occasional outbreaks in humans. Mosquito olfaction plays a significant part in host-seeking behavior of mosquitoes. An evolutionary adaptation of *Ae. aegypti* to human odors which was driven by single nucleotide polymorphisms in the gene encoding an odorant receptor has been presented in a past study. However, genetic alteration in mosquito genome would take longer time, and other possible mechanism of instability and plasticity in host preference of mosquito is to be investigated for prevention of mosquito-borne diseases. We developed genetically modified strains of yellow fever mosquito (*Aedes aegypti*) lacking either odorant-binding proteins 34 or 39 gene by CRISPR/Cas9 system. These mosquitos showed impaired host-seeking activity. It has been known that several species of mosquitoes can experimentally adapt to a new host animal by repeated blood-sucking in a transgenerational manner. For artificially inducing mosquito adaptation to different host species, we are currently establishing *Ae. aegypti* strains reared with mice, rabbits, chickens, common marmosets, and horses as sources of blood meal.

Publications

Chinuki Y*[1]*, Ishiwata K, Yamaji K, Takahashi H*[1]*, Morita E*[1] *(*[1]Shimane Univ).*** Haemaphysalis longicornis tick bites are a possible cause of red meat allergy in Japan. *Allergy.* 2016; **71:** 421-5.

Centers of Advanced Medicine
Center for Medical Science of Fatigue

Hiroyuki Yanagisawa, *Professor and Director*

General Summary

The Jikei Center for Medical Science of Fatigue (JCMSF) was established in 2014 with support from the Ministry of Education, Culture, Sports, Science and Technology–Supported Program for the Strategic Research Foundation at Private Universities. The JCMSF is aimed at contributing to human welfare through developing novel methods for the diagnosis, prevention, and care of fatigue–related diseases. For this aim, our research focuses on the mechanism of fatigue and fatigue–related diseases.

Fatigue is caused by many different factors, including sleep deprivation, persistent mental activity, and prolonged physical exertion. Long–term fatigue is reportedly experienced by at least 50% of workers in Japan and can cause cardiovascular dysfunction, such mental health disorders as depression, and occupational sudden death (*karoshi*).

Fatigue levels are frequently assessed with self–reporting questionnaires of feelings of fatigue, such as the Checklist Individual Strength and the Profile of Mood States, or with visual analog scales. However, negative or positive events at work are associated with the feeling of fatigue, and compensation practices within some industries tend to motivate individuals to distort their self–reported fatigue levels. Therefore, an individual's perception of fatigue may not be a correct indicator of fatigue.

Fatigue is associated with a perception of fatigue mediated by signaling pathways in the central nervous system. The mechanism for perceiving fatigue is thought to be associated with changes in levels of inflammatory cytokines and with changes in the autonomic nervous system. Because no objective measure of fatigue is universally accepted, serum inflammatory cytokine levels and neurobehavioral assays, such as psychomotor vigilance tests, are frequently used as biomarkers for fatigue.

Work–induced fatigue is frequently confused with pathological fatigue, such as chronic fatigue syndrome (CFS). The CFS is triggered by infection rather than overwork, and the diagnostic criteria for CFS are 6 months of unexplained fatigue that is not alleviated by rest and the presence of 4 of 8 additional symptoms (e.g., unrefreshing sleep, sore throat, and muscle pain). The CFS is thought to affect 1 to 8 of every 1,000 adults in the United States. Biomarkers proposed for diagnosing CFS have included cytokines, adrenergic genes, immunological markers, and cortisol. However, most of these markers are common to physiological fatigue, and even with these biomarkers distinguishing CFS and physiological fatigue is difficult.

When JCMSF was established it focused on indentifying biomarkers that could be used to distinguish physiological fatigue from pathological fatigue. We examined the amounts of salivary human herpesvirus (HHV) 6 and HHV–7 due to training in members of JCMSF. Because fatigue scores increased during training, we believed training provided sufficient physiological fatigue loading. The amounts of salivary HHV–6 and HHV–7 DNA

increased with training and decreased with rest, suggesting their usefulness as biomarkers of physiological fatigue. The amounts of HHV-6 and HHV-7 were also correlated with working time; however, they were not reactivated by pathological fatigue. These findings suggest that HHV-6 and HHV-7 are reactivated by physiological fatigue but not by pathological fatigue.

Research Activities

Effects of Nutritional Supplementation on Fatigue, and Autonomic and Immune Dysfunction in Patients with End-Stage Renal Disease: A Randomized, Double-Blind, Placebo-Controlled, Multicenter Trial

Fatigue is a predictor of cardiovascular events in patients with end-stage renal disease (ESRD) undergoing hemodialysis treatment. We hypothesized that multinutritional support would improve quality of life, fatigue symptoms, and potential quantitative measures including endocrine, immune and autonomic functions in patients with ESRD undergoing hemodialysis.

Two hundred and two hemodialysis patients were randomly assigned to receive active treatment (containing vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12, folic acid, vitamin C, carnitine, coenzyme Q10, naïve galacto-oligosaccharide, and zinc) or placebo after each dialysis session for 12 weeks. The patients and attending physicians were blinded to the treatment, and 172 patients (86 in each group) completed the study. Fatigue was evaluated via fatigue questionnaire at 0, 4, and 12 weeks. To assess human herpes virus (HHV) 6 and 7 reactivation, numbers of viral DNA copies were determined in saliva by polymerase chain reaction at weeks 0 and 12. Autonomic function was determined via measurement of beat-to-beat variation by using acceleration plethysmography.

Clinical characteristics, changes in fatigue, quality of life score, endocrine functions, and laboratory data did not differ significantly between the two groups. Several parameters of heart rate variability significantly increased after nutritional treatment compared to placebo. Nutritional drink for 12 weeks significantly suppressed HHV7 DNA copy numbers. Similarly, HHV6 DNA copy numbers tended to be decreased by treatment but without reaching statistical significance.

Nutritional supplementation may modulate immune and autonomic dysfunction in ESRD patients undergoing hemodialysis.

Reduction of adverse effects by a mushroom product, active hexose correlated compound (AHCC) in patients with advanced cancer during chemotherapy—the significance of the levels of HHV-6 DNA in saliva as a surrogate biomarker during chemotherapy

Chemotherapy improves the outcome of cancer treatment, but patients are sometimes forced to discontinue chemotherapy or drop out of a clinical trial due to adverse effects, such as gastrointestinal disturbances and suppression of bone marrow function. The objective of this study was to evaluate the safety and effectiveness of a mushroom product, active hexose correlated compound (AHCC), on chemotherapy-induced adverse effects and quality of life (QOL) in patients with cancer. Twenty-four patients with cancer received their first cycle of chemotherapy without AHCC and then received their second

cycle with AHCC. During chemotherapy, we weekly evaluated adverse effects and QOL via a blood test, EORTC QLQ-C30 questionnaire, and DNA levels of herpes virus type 6 (HHV-6) in saliva. The DNA levels of HHV-6 were significantly increased after chemotherapy. Interestingly, administration of AHCC significantly decreased the levels of HHV-6 in saliva during chemotherapy and improved not only QOL scores in the EORTC QLQ-C30 questionnaire but also hematotoxicity and hepatotoxicity. These findings suggest that salivary HHV-6 levels may be a good biomarker of QOL in patients during chemotherapy, and that AHCC may have a beneficial effect on chemotherapy-associated adverse effects and QOL in patients with cancer undergoing chemotherapy.

Centers of Advanced Medicine
Stable Isotope Medical Application Center

Tomokazu Matsuura, *Professor*
Takashi Okano, *Professor*
Koji Nakada, *Associate Professor*
Takeo Iwamoto, *Professor*
Koji Takada, *Professor*
Youichiro Kusakari, *Associate Professor*

General Summary

In the fundamental research, we started the study using the diabetes model rat. About fasting ^{13}C-glucose breath test (FGBT), we performed the proof experiment using the model animal. We observed the process when liver insulin resistance is brought about by animal model of diabetes mellitus.

As clinical studies, we promoted the practical use study of the stable isotope breath test.

1. A liver insulin resistance evaluation by FGBT for circulatory diseases, and diabetes mellitus.
2. Promoted the practical use of the examination for ^{13}C breath test for stomach discharge function (simple method).

Publications

Konishi H, Nakada K, Kawamura M, Iwasaki T, Murakami K, Mitsumori N, Yanaga K. Impaired Gastrointestinal Function Affects Symptoms and Alimentary Status in Patients After Gastrectomy. *World J Surg.* 2016; **40:** 2713-8.

Nakada K, Takahashi M[1], Ikeda M[2], Kinami S[3], Yoshida M[4], Uenosono Y[5], Kawashima Y[6], Nakao S[7], Oshio A[8], Suzukamo Y[9], Terashima M[10], Kodera Y[11] ([1]Yokohama Municipal Citizen's Hosp, [2]Asama General Hosp, [3]Kanazawa Med Sch, [4]International Univ Health Welfare Hosp, [5]Kagoshima Univ, [6]Saitama Cancer Center, [7]Tokyo Women's Med Univ, [8]Waseda Univ, [9]Tohoku Univ, [10]Shizuoka Cancer Center, [11]Nagoya Univ). Factors affecting the QOL of patients after gastrectomy as assessed using the newly developed PGSAS-45 scale: a nationwide multi-institutional study. *World J Gastroenterol.* 2016; **22:** 8978-90.

Sakaguchi M[1], Manabe N[2], Ueki N[3], Miwa J[4], Inaba T[5], Yoshida N[6], Sakurai K[7], Nakagawa M[8], Yamada H[9], Saito M[10], Nakada K, Katsuhiko Iwakiri K[11], Joh T[12], Haruma K[2] ([1]Moriguchi Keijinkai Hosp, [2]Kawasaki Med Sch, [3]Tokyo Rosai Hosp, [4]Toshiba General Hosp, [5]Kagawa Prefectural Central Hosp, [6]Jpn Red Cross Kyoto Daiichi Hosp, [7]Hattori Clinic, [8]Hiroshima City Hosp, [9]Shinko Hosp, [10]Michiya Clinic, [11]Nippon Med Sch, [12]Nagoya City Univ). Factors associated with complicated erosive esophagitis: A Japanese multicenter, prospective, cross-sectional study. *World J Gastroenterol.* 2017; **23:** 318-27.

Takahashi M[1], Terashima M[2], Kawahira H[3], Nagai E[4], Uenosono Y[5], Kinami S[6], Nagata Y[7], Yoshida M[8], Aoyagi K[9], Kodera Y[10] ([1]Yokohama Municipal Citizen's Hosp, [2]Shizuoka Cancer Center, [3]Chiba Univ, [4]Kyushu Univ, [5]Kagoshima Univ, [6]Kanazawa Med Sch, [7]Nagasaki Univ, [8]International Univ Health Welfare Hosp, [9]Kurume Univ, [10]Nagoya Univ), Nakada K. Quality of life after total vs distal gastrectomy with Roux-en-Y reconstruction: Use of the Postgastrectomy Syndrome Assessment Scale-45. *World J Gastroenterol.* 2017; **23:** 2068-76.

Centers of Advanced Medicine
Center for Biofilm Science and Technology

Yoshimitsu Mizunoe, *Professor and Director*
Kazuhiro Hashimoto, *Professor*
Keishi Marumo, *Professor*
Shin Egawa, *Professor*
Koji Takada, *Professor*
Tetsuya Horino, *Associate Professor*
Noriyuki Murai, *Assistant Professor*
Midori Kono, *Assistant Professor*
Tadayuki Iwase, *Assistant Professor*
Ken-ichi Okuda, *Assistant Professor*
Seiji Hori, *Professor*
Katsuhiko Yanaga, *Professor*
Shoichi Uezono, *Professor*
Takeo Iwamoto, *Professor*
Ken Kaito, *Professor*
Jun Araya, *Associate Professor*
Ryuichi Nagahori, *Assistant Professor*
Akiko Tajima, *Assistant Professor*
Shinya Sugimoto, *Assistant Professor*

General Summary

The Jikei Center for Biofilm Science & Technology (JCBST) was established in April 2015 as a member of the Centers of Advanced Medicine of The Jikei University with the support of the Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Strategic Research Foundation at Private Universities. The JCBST will promote research for the prevention and control of biofilm-associated infections. Research projects of the JCBST have focused on: 1) identification of ABC-JK2, a small molecular inhibitor of staphylococcal biofilm formation, 2) exploration of novel physiological functions of polyphenols, 3) imaging of biofilms in solution by atmospheric scanning electron microscopy, 4) importance of extracellular RNA in bacterial biofilms, 5) genotypic and biofilm profiles of *P. acnes* isolated from pacemakers without clinical signs of infection, 6) redundancy and complexity in biofilms.

Research Activities

Identification of ABC-JK2, a small molecular inhibitor of staphylococcal biofilm formation

In this study, we aimed to identify compounds that inhibit biofilm formation by S. aureus. One of the hit compounds, named anti-biofilm-compound JK2 (ABC-JK2), inhibited biofilm formation of several strains of S. aureus including MRSA and S. epidermidis at IC50 range of 12.0 to 22.5 μM. Metabolomic analysis showed that ABC-JK2 decreases intracellular levels of glycolytic metabolites. Microarray/quantitative real-time PCR revealed up-regulation of genes related to peptidoglycan biosynthesis and hydrolases. In addition, TEM observation showed that bacterial cell wall thickness and number of abnormal septa are increased in the presence of ABC-JK2. It was suggested that ABC-JK2 inhibits staphylococcal biofilm formation by affecting glycolysis and cell wall synthesis.

Exploration of novel physiological functions of polyphenols

Exploration of potential functions of food constituents provides an additional value for

health as well as offers applications for preventing diseases. In this study, we identified myricetin (Myr), a kind of polyphenol produced by plants, to effectively prevent biofilm formation by E. coli and S. aureus including methicillin-resistant strains, in a dose-dependent manner. In addition, a more effective Myr-derivative with approximately 10-fold higher activity than Myr was identified. Its mode of action is now elucidated.

Imaging of biofilms in solution by atmospheric scanning electron microscopy
In this study, we visualized biofilms immersed in aqueous solution, including biofilms formed by the Gram-positive coccus Staphylococcus aureus and the Gram-negative bacillus Escherichia coli by recently developed atmospheric scanning electron microscopy (ASEM). Since ASEM allows a biofilm cultured on electron-transparent film windows to be observed by an inverted SEM from below, it was possible to study biofilm formation near the substrate and the ECM at high resolution. Membrane vesicles, delicate spiral flagella, straight curli fibres, and filamentous extracellular DNA networks were observed by ASEM with labelling methods such as labelling with positively charged Nanogold, heavy metals, and immuno-gold. Collectively, our results suggest that ASEM is a broadly applicable approach for microbial research and diagnostic purposes.

Importance of extracellular RNA in bacterial biofilms
We recently explored presence of extracellular RNA (eRNA) in Staphylococcus aureus biofilms. In this study, we analyzed its roles in biofilm development. The molecular size of the eRNA was estimated 20 to 100 nucleotides by urea-denaturing acrylamide gel electrophoresis. We observed localization of eRNA in the three-dimensional structure of biofilm by confocal laser scan microscopy. In addition, degradation of polysaccharides, which are major components of S. aureus biofilms, induced the release of eRNA from the biofilm. Furthermore, we demonstrated by surface plasmon resonance that purified polysaccharides bound to eRNA, indicating that polysaccharides are important to maintain eRNA in the biofilm. Our findings provide evidence of a novel function for RNA that has important implications for understanding biofilm physiology.

Genotypic and biofilm profiles of P. acnes *isolated from pacemakers without clinical signs of infection*
Colonization of bacteria on the surfaces of cardiac pacemakers explanted from patients without clinical evidence of infection was consecutively analyzed. P. acnes was isolated from pacemakers without clinical signs of infection at high frequency (23%). Biofilm forming capacities of the P. acnes isolates and biochemical properties of the biofilms were different among strains regardless of the STs, however, extracellular DNA was suggested to be a factor commonly involved in biofilm formation of diverse P. acnes strains. High-resolution observation of nanostructures in the biofilms by transmission electron microscopy and atmospheric scanning electron microscopy visualized cytoplasmic components leakage along with cell lysis and fiber structures connecting cells in biofilm.

Redundancy and complexity in biofilms
Biofilm-forming capacity is determined by a self-produced extracellular matrix (ECM).

Our recent studies demonstrated that MR23, a clinical isolated strain of methicillin-resistant S. aureus, forms a robust proteinaceous biofilm. In addition, extracellular adherence protein (Eap), an S. aureus-specific secreted protein, was found to be abundant in ECM and to promote biofilm formation by S. aureus. However, deletion of the eap gene did not affect biofilm formation, suggesting the presence of other genes responsible for biofilm formation in MR23. To address this, a number of single and multiple gene-deletion mutants were constructed. Interestingly, simultaneous deletion of eap and sasG encoding a cell wall-anchored protein reduced biofilm formation, but single deletion of sasG did not. These results suggested that Eap and SasG have an overlapping function each other. Elucidation of their roles in biofilm formation may lead to the development of specific treatment for S. aureus infections.

Publications

Sugimoto S, Okuda K, Miyakawa R, Sato M[1], Arita-Morioka K[2], Chiba A, Yamanaka K[2], Ogura T[2], Mizunoe Y, Sato C[1] ([1]AIST, [2]Kumamoto Univ). Imaging of bacterial multicellular behaviour in biofilms in liquid by atmospheric scanning electron microscopy. *Sci Rep.* 2016; **6:** 25889.

Iwase T, Ogura Y[1], Hayashi T[1] ([1]Kyushu Univ), Mizunoe Y. Complete Genome Sequence of Klebsiella pneumoniae YH43. *Genome Announc.* 2016; **4:** pii: e00242-16.

Iwase T, Ogura Y[1], Hayashi T[1] ([1]Kyushu Univ), Mizunoe Y. Complete Genome Sequence of Klebsiella oxytoca Strain JKo3. *Genome Announc.* 2016; **4:** pii: e01221-16.

Clinical Research Support Center

Shigeru Kageyama, *Professor and Director* Masako Nishikawa, *Professor*

General Summary

The Clinical Research Support Center was founded in April 2014 to promote the proper conduct of clinical research. The center has the following functions: protocol planning, statistical analysis, monitoring, support for clinical research conduct, and education. We started consulting for clinical research in September 2014 and had 40 protocols of consultation from April 2016 through March 2017. Consultations were as follows: 20 protocols for protocol planning and statistics (objective of the research, study design, control arm, study participants and their recruitment method, randomization, primary endpoint and its rationale, procedure to avoid/reduce bias, data collection, stopping criteria, statistical analysis, analysis sets, and sample size calculations), 5 protocol for the statistical analyses,1 protocol for the preparation of article, 8 protocols for the response to reviewers after the submission of articles (including additional analyses), 4 protocols for application of AMED research grant, and 11 protocols for conducting statistical analysis. Consultations were requested by the Departments of Endoscopy, Psychiatry, Cardiovascular Surgery, Anesthesiology, Neurosurgery, Otorhinolaryngology, Radiology, Pediatrics, Ophthalmology, Urology, Radiology, Surgery (Division of Breast and Endocrinology Surgery; Vascular Surgery), and Internal Medicine (Divisions of Diabetes, Metabolism and Endocrinology; Clinical Oncology/Hematology; Gastroenterology and Hepatology; and Nephrology and Hypertension), ICU, Laboratory Medicine, Tropical Medicine, Research Center for Medical Sciences, Center for International Affairs and students of the nursing master's degree course.

In cooperation with the Division of Clinical Pharmacology and Therapeutics we held a "Clinical Trial Seminar" 2 times to improve literacy about clinical trials among researchers. The themes were "Systems for improving the quality of clinical trials" "Basic knowledge on intellectual property for researchers". We also held a "Biostatistics Seminar for Tomorrow" consisting of 2 basic courses and 2 advanced courses to promote appropriate trial designs and the application of biostatistical methods.

Ethical guidelines for medical and health research involving human subjects have been implemented since April 2015. In these guidelines the principal investigator is obliged to perform monitoring if interventional studies are invasive. To meet this demand we prepared standard operating procedures for monitoring. The monitoring is performed by clinical research coordinators themselves or by supported investigators.

We introduced a clinical research liaison system to facilitate clinical research. We requested departments conducting many clinical trials to assign liaison physicians. Liaison physicians are invited to participate with priority in the "Biostatistics Seminar for Tomorrow" and are expected to act as liaisons between the department to which they belong and the Clinical Research Support Center.

Research Activities

Statistical methods of analyzing competing risks data

In the analysis of survival data, an individual is subjected to an event due to only 1 of several distinct types of causes, and the occurrence of 1 type omits other types of causes, such as death due to stroke and death due to myocardial infarction. These event types are given the statistical term "competing risks." When the primary endpoint is the mean change/percent change of a variable, such as HbA1c or blood pressure, from the baseline to the planned end of the study and is repeatedly measured, a typical problem is missing data. Nowadays, intensive discussions are done about the problem of missing data, and an Addendum to the Statistical Principles for Clinical Trials of the International Conference on Harmonization has been started by its expert working group. We have considered study design and methods of statistical evaluation applying a method of analyzing data on competing risks in such a situation since last year. We improved the methods of statistical evaluation from the point of beneficial effect for patients in non-inferiority trials.

To evaluate within patient consistency between measures, for example, pain intensities of patients are repeatedly measured with a visual analogue scale and an objective measuring device (Pain Vision, Nipro Co., Osaka) in clinical research, intraindividual coefficient of variations are compared between measures. The correlated samples, different interindividual variations due to different scales of the measures and missing data in either measure in certain time points should be taken into account in statistical analysis. In such a situation, a statistical approach to compare the intraindividual coefficient of variations was proposed with the adjustment of covariates.

Publications

Odawara M*[1], *Kawamori R*[2], *Tajima N, Iwamoto Y*[3], *Kageyama S, Yodo Y*[4], *Ueki F*[4], *Hotta N*[5] ([1]*Tokyo Medical Univ,* [2]*Juntendo Univ Grad Sch Med,* [3]*Asahi Life Foundation,* [4]*Sumitomo Dainippon Pharma,* [5]*Chubu Rosai Hosp).

Long-term treatment study of global standard dose metformin in Japanese patients with type 2 diabetes mellitus. *Diabetology Int.* 2017; **8:** 286-95. Epub 2017 Feb 24.

Premedical Course

Biology

Koji Takada, *Professor* Rie Hiratsuka, *Associate Professor*

General Summary

Our research themes are to understand the mechanism of protein aggregate formation associated with heavy metal cytotoxicity, and the mechanism of generative cell migration in vegetative cell of angiosperm pollen.

Research Activities

Analysis of cadmium-induced cellular protein aggregation

Cadmium is a toxic heavy metal preferentially accumulated in renal cortex of the mammals. One of possible reasons for cadmium cytotoxicity is radical-dependent cellular damage. Ubiquitin-mediated proteolysis has a protective role against the cadmium cytotoxicity. We have found that sublethal cadmium exposure to human kidney HK-2 cells markedly increases amounts of polyubiquitin-containing protein aggregates preceding the cell death. The process of such aggregate formation was analyzed based on the method of stable isotope labeling by amino acids in cell culture (SILAC). Under the condition of SILAC experiments, the sublethal Cd exposures ($\geqq$ 20 μM for 24 h) accumulated polyubiquitinated proteins in the fraction containing denatured proteins insoluble with 1% Triton X-100 (Triton insoluble protein fraction). ^{13}C-amino acids were incorporated into both Triton-soluble native proteins and Triton-insoluble denatured proteins during cadmium exposure, and a prolonged exposure brought a relative decrease of the ^{13}C ratios in the Triton-insoluble protein fractions, suggesting a chronic cadmium exposure may damage metastable cellular proteins than nascent proteins.

Ultrastructural analysis of microgametogenesis in rice

In angiosperm pollen, intracellular migration of generative cell into the vegetative cell cytoplasm is one of the important mechanisms to ensure pollen tube fertilization. In this study, three strains of rice mutants(#0113, #0354, #0365) in which migration of the generative cell is blocked were analyzed using electron microscopy. The heterozygotes of these mutants produced approximately equal numbers of normal and abnormal pollen. At the heading stage, many lipid bodies were distributed in all 3 abnormal pollen. The single large vacuole and small sized starch granules were observed within the vegetative cell. On the other hand, specific abnormalities were also observed in each mutant. In abnormal pollen of #0354, the cell wall between generative and vegetative cell was markedly thickened compared to normal pollen and abnormal pollen of other lines. In abnormal pollen of #0365, generative cell membrane was spread in an ameboid shape. Based on the results above, the causative genes of three strains were pollen expressing gene, which was

expected to be different for each strain.

Physics

Tsuyoshi Ueta, *Professor* Katsumi Kasono, *Assistant Professor*

General Summary

1. We have proposed a disordered air rod photonic crystal as a model of a sponge structure inside a barb and have confirmed that the color of birds, such as the and the red-flanked bluetail, is a structural color owing to the interference of the light within a barb by reproducing the reflection spectrum.
2. We have investigated the effect of optical absorption on the radiation of electromagnetic waves in excited states from an artificially vibrated photonic crystal and have found that it inconceivably even enhances the intensity of higher mode radiation.
3. We are studying an ultrasonic lens with an actively deformable phononic structure constructed with micro-tubes into which liquid metal is injected. In this research, we are attempting to stimulate a cerebral deep part by designing a phononic lens in which a brain and the cranial bones are also taken into account as metamaterials.
4. We have been studying computational methods and algorithms for condensed matter theory. The phenomena interested in are phase transitions and critical phenomena.

Research Activities

1. The concept of topology-optimized carpet cloaks is investigated by using level-set boundary expressions. Specifically, these carpet cloaks are designed with the idea of minimizing the value of an objective functional, which is here defined as the integrated intensity of the difference between the electric field reflected by a flat plane and that controlled by the carpet cloak.
2. We have so far investigated the optical properties within a one-dimensional photonic crystal whose stacked metallic plates are artificially driven by using actuators. In the present study, we compute the wave functions and phase shifts between the transmission and reflection coefficients around the amplifying resonance, and approach and discuss the resonance conditions with the photonic band structures and the Friedel sum rule in one dimension. We have investigated also a disordered photonic crystal case, which is metal plates of random thickness arranged in parallel with equi-intervals.
3. A crystal-growth based model taking into consideration the microscopic process of the proliferation of cancer cells is proposed, and a parameter domain in which the structure of the cancer cell cluster agrees with experimentally observed one has been found out. The shape variation of the cancer cluster has been acquired using the proposed model by changing the nutrient level, the ease of the proliferation and the mobility of cells.
4. We have made Multigrid cluster Monte Carlo simulations to study q-state Potts models

on the square lattices with ferromagnetic phase transitions. We calculated relaxation time of order parameter.

Publications

Fujii G, Ueta T. Topology-optimized carpet cloaks based on a level-set boundary expression. *Phys Rev E.* 2016; **94:** 043301.

Chemistry

Takashi Okano, *Professor*

Naruyoshi Komiya, *Associate Professor*

General Summary

The research of this laboratory is focused on synthesis-oriented organic chemistry, including the synthesis of bioactive compounds and fluorine-containing materials, and the development of novel functional organometallic compounds, including the highly emissive phosphorescent materials in solid state and kinetic probe for dynamic behavior in solution state.

Research Activities

Preparation of Fluorine-containing organic compounds via NHC Catalysis

NHC (N-heterocyclic carbene) is a highly stable carbanion stabilized by the aromatic azolium system including vitamin B_1. NHC is now attracting interests as an environmentally compatible organic catalyst for the various reactions using aldehydes as acyl anion equivalents. However, the NHC reaction of trifluoroacetaldehyde (TFA) is not expected to be simply applied as aliphatic aldehydes because of the anomalous character of fluorine. As a preliminary study, the NHC reaction of TFA was examined theoretically. The calculation predicted successfully the NHC reaction of TFA is possible even though the dehydration from the TFA hydrate, usual state of TFA, is necessary.

Kinetic Studies of the Chirality Inversion of Salicylaldiminato-Ruthenium Using Racemic η^6-p-Cymene Complexes as a Mechanistic Probe

Kinetic studies of the chirality inversion of a series of mono- and bimetallic (*p*-cymene)(salicylaldiminato)Ru(II) complexes with halo ligands in solution have been performed by means of the line-shape method, using ^{1}H NMR spectroscopy to evaluate the signal exchange rates between diastereotopic protons in the *p*-cymene ligand. The activation parameters ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) associated with the flipping molecular mobility were determined from variable-temperature NMR analyses, and it was found that the neutral halo complexes exhibit much lower enthalpies and entropies than the corresponding cationic pyridine analogues.

Publications

Komiya N, Nakajima T[1], Hotta M[1], Maeda T[1], Matsuoka T[1], Kawamorita S[1], Naota T[1] ([1]Osaka Univ). Kinetic studies of the chirality inversion of salicylaldiminato ruthenium using racemic η^6-*p*-cymene complexes as a mechanistic probe. *Eur J Inorg Chem.* 2016; 3148-56.

Naito M[1], Komiya N, Naota T[1] ([1]Osaka Univ). Homochiral association behavior of binuclear *trans*-bis(*β*-iminoaryloxy)palladium(II) complexes doubly linked with *m*-xylylene spacers: Drastic linker-dependence of the association chirality of chiral clothespin-shaped molecules. *Org Chem Front.* 2016; **3:** 3148-56.

Hashimoto T[1], Fukumoto K[1], Ngoc Ha-Thu Le[1], Matsuoka T[1], Kawamorita S[1], Komiya N, Naota T[1] ([1]Osaka Univ). Dynamic neighbouring participation of nitrogen lone pairs on the chromogenic behaviour of *trans*-bis(salicylaldiminato)Pt(II) coordination platforms. *Dalton Trans.* 2016; **45:** 19257-68.

Social Science (Law)

Ryuichi Ozawa, *professor*

General Summary

Problems of Constitutional Law in present-day Japan.

Research Activities

OZAWA published Articles and Books cited n Japanese Research Activities 2016.

Human Science

Kazushi Misaki, *Professor*

General Summary

The Study of Western philosophy and ethics.

Research Activities

Origin of the ego; The intersubjective approach to the subject

Descartes' "cogito", the ego as subject of thought, is still a popular and paradigmatic image for the human subjekt: to be a mature human means that one can think independently and autonomously and one can act according to the belief of his own.

In the modern philosophy this image of the ego has been attacked from various positions. One of those, an intersubjective approach criticizes Descartes' cogito as isolated subject and maintains that an ego can be a subject only in the intersubjective relations. Trough the recognition of the othes one can become and can be a subject. Studies by Donald Winnicott show how important the relationship of the baby with his mother is at the first stage

of the ego. George Herbert Mead considers the development of the ego as a process of 'ideal roll-taking of others'. The goal of this development is the subjekt that can think from the universal point of the view, as Descartes imagined.

Learn from the experience in Auschwitz
From anather respect the 'inhuman' situations in the concentration camp Auschwitz show vorious elements needed to be 'human'. From the experience written by Frankl in Auschwitz we can learn the 'human conditions' that in ordinary life remain unconscious but essential.

Japanese

Ikuko Noro, *Professor*

General Summary

A study on elderly patients' comprehension of information during Informed Consent
I investigated how the comprehensibility of the physician's verbal explanation as well as his attitude during informed consent affected the comprehension of the information and decision-making in elderly patients.

Research Activities

I presented the results at the symposium of 31st International Congress of Psychology 2016.

Mathematics

Katsuya Yokoi, *Professor*
Yasuko Hasegawa, *Assistant Professor*

General Summary

1. To study dimension theory and topological dynamics
2. To study construction of automorphic forms in several variables

Research Activities

1. We studied omega-limit sets, (strong) chain recurrent sets on topological dynamics, Conley index theory, and LS-category.
2. The Fourier expansion of Eisenstein series is important research in the theory of num-

bers. We gave an explicit formula of a spherical function on GL(3,R) which is a part of the research.

English

Osamu Ohara, *Professor* Tetsuro Fujii, *Associate Professor*

General Summary

English Language communication and education: material analysis and development (Fujii)

Fujii joined a project team to compile English textbooks for high school English classes: *English Communication I, II, and III*. Along with compiling the textbooks, Fujii has been writing their exercise materials and teacher's manuals. In addition, Fujii has been studying how teaching materials influence learner motivation and language development.

Research Activities

Fujii analyzed and collected authentic English materials to meet the level and the needs of high-school textbooks based on current teaching methods, theories, and research findings on learning English as a foreign language. These materials were used to compile textbooks following the revised teaching guidelines set out by the Ministry of Education, Culture, Sports, Science and Technology. New edition of the textbook, *World Trek English Communication I*, was officially approved by the Ministry and published in February 2017.

Fujii presented about the effects of English class materials that were created to improve learner's reading speed and knowledge of words in "Practical application of teaching materials that integrate fast-reading comprehension and vocabulary acquisition" at Japan Association of College English Teachers (JACET) Joint Forum of Reading Study Group and English Dictionary Research Group in Waseda University, Tokyo in March 2017.

Reviews and Books

Mochizuki M[1], Aizawa K[2], Allum P[3], Sasabe N[4], Hayashi Y[5], Fujii T, Miura S[6] ([1]Reitaku Univ, [2]Toyo Denki Univ, [3]Rikkyo Univ, [4]Toritsu Aoyama High, [5]Soka High, [6]Tsurubunka Univ). World Trek: English Communication I. Tokyo: Kirihara Shoten; 2017.

Mochizuki M[1], Aizawa K[2], Allum P[3], Sasabe N[4], Hayashi Y[5], Fujii T, Miura S[6] ([1]Reitaku Univ, [2]Toyo Denki Univ, [3]Rikkyo Univ, [4]Toritsu Aoyama High, [5]Soka High, [6]Tsurubunka Univ). World Trek: English Communication I. Teacher's Book. Tokyo: Kirihara Shoten; 2017.

First Foreign Languages

Katsumi Suzuki, *Associate Professor*

General Summary

German contemporary literature.

Research Activities

The research topic: “the modern German literature of nonnative writers in German-speaking areas”.
At a symposium, where I participated as a panelist and gave a presentation about Rafik Schami who is a famous German writer, coming from Syria, and his latest novel “Sophia, I got to know the name of the writer who hat background as migrants, Sherko Fatah. His father is Kurdish Iraqi and his mother is Polish German. I am working now with his novels and translated a great novel “ein weißes Land (a white country)” into Japanese.

School of Nursing

Basic Nursing

Sachiko Tanaka, *Professor*
Chieko Hanyu, *Assistant Professor*
Noriko Aoki, *Assistant Professor*
Mayumi Kikuchi, *Associate Professor*
Sumiko Satake, *Assistant Professor*

General Summary

Major study areas in basic nursing include: 1) education on physical assessment and supporting techniques, 2) supporting techniques in daily living, 3) the history of nursing, 4) supporting patients with progressive motor dysfunction, and 5) nursing diagnosis.

Research Activities

Sachiko Tanaka: Tanaka studied Healthy Work Environment of Nurses who Working in Hospitals.
Mayumi Kikuchi: To review the methods of nursing practice for patients with progressive motor dysfunction, Kikuchi qualitatively described how nurses working in a ward for patients with muscular dystrophy should handle clinical situations.
Chieko Hanyu: Hanyu performed a questionnaire survey of teachers to understand the current status of education on physical assessment accompanying the revision of the 2009 curriculum.
Sumiko Satake: Satake studies it under the theme of "a reply of an emotion change and the autonomic nerve activity to hearing stimulation in the long-term lying in bed patient." In addition, she has worked as a research member of the "Literature Review Complex Feelings of Patients with Acute Aphasia".
Noriko Aoki: Aoki studied easing intra-abdominal pressure by changing the head elevation angle while the patient uses a bedpan.

Nursing Administration

Midori Nagano, *Professor*

General Summary

Two studies have been performed "Relating factors of assistant-dependent replacement of stoma appliances and peristomal skin irritations associated with ostomy from rectal cancer" and "Health Work Environment in Nursing Practice".

Research Activities

Relating factors of assistant-dependent replacement of stoma appliances and peristomal skin irritations associated with ostomy from rectal cancer
I examined the appropriate support from findings to an ostomate corresponding to the Aging in patients and the improved Chemotherapy using documents. And I submitted it to the St. Luke's international university as a doctoral dissertation, and Nagano became recognized as an article doctor in September. I announced the complications with ostomy surgery and peristomal skinirritation at academic meetings.

Health Work Environment in Nursing Practice
I supported other researchers announced it in societies.

Adult Nursing

Masami Sato, *Professor*
Mai Hosokawa, *Assistant Professor*
Wakako Osaka, *Assistant Professor*
Ruka Seyama, *Associate Professor*
Yoko Murooka, *Assistant Professor*

General Summary

Undergraduate students were offered classroom coursework including an introduction to clinical nursing and four areas of clinical nursing based on health level (chronic phase, perioperative period, cancer and acute phase). An educational evaluation was conducted with emphasis on the process of learning practical nursing skills through chronic phase and perioperative nursing practicum. As part of their research activities, each of the faculty members explored cancer nursing topics as well as nursing care for acute and critically ill patients.
Based on educational evaluation of the past, was established from the 2012 fiscal year the new curriculum, which was offered 3rd year of "adult nursing practice theory" that the purpose of critical thinking competency building. We do not have enough teacher member this year, then we change class schedules and teaching methods, through cooperation and partnership. More advanced to work using the video material and designed thinking in realistic situations, made its own case information in the paper is not to collect information, develop a nursing plan. Teaching methods include group learning based on PBL of interrelated ways. We impose assignment to individual for effective learning on PBL. Learning assessment perspective is evaluated by each faculty member along with it, create a Rubric and numerous meetings with groups of students. The students discussed though poster presentation. Many students participated proactively, but small number of students could not. Learning attitude was different by the student. Teaching evaluation by students was generally positive in the class schedule change, long empty between classes that was challenges from the viewpoint of learning continuity.

While student evaluations of nursing process development, which included information collection and nursing practice utilizing nursing plans, were largely positive for the overall adult nursing practicum, those of faculty members tended to be lower. In the practicum environment and organizational arrangements, cooperation with clinical practicum instructors was strengthened by setting up opportunities for students to review the work with their instructions. Students generally had positive evaluations of educational interventions by the faculty members, such as faculty being present at the clinical scene and providing advice and critique in a timely manner, providing clues on nursing process development based on records, conducting nursing practice together with the students, and ensuring safety. These are aspects that we hope to continue, and practicum training with appropriate interactions is also anticipated in the future by adjusting the division of roles of the parties involved.

Research Activities

Research on Cancer Nursing

1. Research of nursing care for rectal cancer undergoing anterior resection

We have been developing nursing how to reduce bowel disorders characteristic of anterior resection.

This year the embarked on a study to investigate validity and discriminative validity was developed to evaluate the effect of nursing intervention on “Defecation Disorder Assessment Scale; DDAS ver.2” validity.

2. Research on the chemotherapy‐induced peripheral neuropathy

As joint research with other facilities, we have been developing the patient educational applications of the chemotherapy‐induced peripheral neuropathy. This year, we have investigated the usefulness of the developed application and clarified that certain effects can be obtained.

3. Research on the cancer patient who receives for the first time outpatient chemotherapy

As joint research with clinical nurse, we have found out the concerns of cancer patient who receives for the first time outpatient chemotherapy and have investigated for support need. This year, we have interviewed 12 patients. We will continue to collect date and analyze in the future.

4. Research on cancer nursing consultation outpatient

As joint research with clinical nurse, we have analyzed the details of the consultation contents of 91 subjects who used cancer nursing consultation outpatient to examine the issues. As a result, it became clear that “suffering to lose life” and “harshness of not being able to self‐manage uneasy feelings”. We will consider systems to support cancer patients and families in outpatient, hospital, and community.

5. An Analysis of Anxiety in Nurses Working in Designated Regional Cancer Care Hospitals That Also Serve as Central Hospitals for AIDS Treatment over Nursing Care Tasks Performed for HIV‐Infected Patients

The results of this study were published in the Journal of the Japan AIDS Society.

The results of the survey revealed that nurses who had never experienced care of HIV positive patients had greater anxiety about care than experienced nurses.

Research on a critical care

1. Analysis of the perioperative pressure ulcer development situation and related factor

As a result of having analyzed the perioperative pressure ulcer development situation and the related factor, redness was found in 14 of 141 subjects (4.84% of incidents). The facter of redness was diagnosis and treatment department (orthopedics), operative time, anesthesia time, posture (perineal position), changing position, preoperative Hb, postoperative Hb, postoperative Alb. Furthermore, the factors of high independence were surgical time and posture.

2. Development of the Japanese version of the International Patient Decision Aids Standards Collaboration Checklist and Instrument

The International Patient Decision Aids (IPDAS) Collaboration has developed a checklist and an instrument (IPDASi v3.0) to assess the quality of patient decision aids in terms of their development process and shared decision-making design components (Joseph et al., 2014). Osaka and research members who belongs other university have been developing of the Japanese version of IPDASi v3.0 based on the five steps described by Beaton. Forward and back translations of the original tool were carried out. The final version will be released on the Internet after applying to IPDAS Collaboration.

Publications

Osaka W, Nakayama K. Effect of a decision aid with patient narratives in reducing decisional conflict in choice for surgery among early-stage breast cancer: A three-arm randomized controlled trial. *Patient Educ Couns.* 2017; **100:** 550-62.

Gerontological Nursing

Fumiko Kajii, *Professor*

Junko Kusachi, *Associate Professor*

General Summary

The following five studies were performed in 2016: 1) The development and evaluation of a fall-detection application and support program for elderly persons, to detect falls and monitor fall-prevention methods, respectively. This work was supported by a Grant-in-Aid for Scientific Research (B). 2) The development of an educational program to train unemployed and retired nurses to support community-dwelling persons with dementia and their family caregivers. This work was supported by a Grant-in-Aid for Challenging Exploratory Research. 3) The effect of seat-cushion position on lower-limb edema and blood flow among wheelchair-bound elderly persons requiring seat-position support. This work was supported by the Nursing school Research Expenses (NRE). 4) The structure of at-home nursing support for elderly persons with dementia, eating disorders, dysphagia, and malnutrition. This work was supported by the NRE. 5) The cahange on the HbA1c score and health action through use of the specimen measurement device. This work was supported by the NRE.

Research Activities

Study 1: The development and evaluation of a fall-detection application and support program for elderly persons, to detect falls and monitor fall-prevention methods, respectively.
We delivered the fall-prevention lecture to 37 elderly people in the control group, aged more than 65 years, three times every other week. We collected data on participants' mental and physical health condition (i.e., BMI, bone density, grip power, eye-opening single-foothold duration, 10 m walk duration, MMSE, and GDS) and health actions (i.e., exercise frequency and social activities) at the first lecture, three months thereafter, and six months thereafter. We are currently analyzing the data.
Study 2: The development of an educational program to train unemployed and retired nurses to support community-dwelling persons with dementia and their family caregivers.
We conducted a mail survey targeting 6,692 graduates of Jikei University, in the school of nursing and the nursing school. Data were collected from 1,905 people (the response rate 28.5%). We are currently analyzing the data.
Study 3: The effect of seat-cushion position on lower-limb edema and blood flow among wheelchair-bound elderly persons requiring seat-position support.
We compared the effect of different wheelchair cushions on lower-limb edema and blood flow in an elderly subject with hemiplegia. The results were presented at the Japan Academy of Nursing Science.
Study 4: The structure of at-home nursing support for elderly persons with dementia, eating disorders, dysphagia, and malnutrition.
We conducted an interview-based study to structure the support provided by home-visiting nurses to dementia-afflicted elderly persons with eating disorders, dysphagia, and malnutrition. Partial results were presented in a nursing research meeting at Jikei University.
Study 5: The cahange on the HbA1c score and health action through use of the specimen measurement device.
We administered self-report questionnaires to 60 users of the specimen measurement device, to clarify continuation of use it, change in HbA1c levels, change in health awareness and action, the frequency of medical consultation, and participation in health instruction for half a year.

Mental Health and Psychiatric Nursing

Takeshi Katsuki , *Professor* Junko Ishikawa, *Assistant Professor*

General Summary

Firstly, we perform lectures to teach students medical systems and social resources based on the mental health and welfare acts. Secondly, we perform lectures to teach them the methods to assess the patients with mental problems on the treatment process.

Research Activities

We have investigated the mental effects of the Great East Japan Earthquake on the general population in Japan for five years. Our research had been supported by a Grant-in-Aid for Challenging Exploratory Research. We examined mental effects among the public throughout Japan 18 months after the Great East Japan Earthquake and attempted to clarify significant factors affecting mental outcomes. We examined outcomes of the Impact of Event Scale-Revised and the 30-item General Health Questionnaire. Multivariate logistic regression was used to calculate the odds ratios and 95% confidence intervals after controlling simultaneously for potential confounders. We have analyzed new data in 2016. Significant factors for mental health problems after the Great East Japan Earthquake were clarified as sleep disorder among over 65 years population in high risk areas for the next great earthquake and tsunami disaster.
Thus, we presented an interim report at the Karei Kenkyukai of Tokyo Women's Medical University in Tokyo.
Moreover, we are researching the human caring approach and have continued to perform discourse analysis.

Child Nursing

Kinu Takahashi, *Pofessor* Michie Nagayoshi, *Assistant Professor*

General Summary

The lectures given to undergraduates included an introduction, methodology and practice, and educational evaluation. This lectures promoted the learning of practical abilities in pediatrics, through training in the inpatient ward, outpatient ward, and the neonatal intensive care unit of The Jikei University and Child Development Center. These educational methods were used to enhance the advocacy of children's rights in clinical situations and to deal with, practice, and learn nursing skills. The students experienced the nursing of children with disease at an acute stage and learned family centered care, around-the-clock care in a multidisciplinary team on the practical training for pediatric nursing.

Research Activities

The process of pediatric nurses to achieve practice that advocates children's rights
This inductive qualitative study aimed to clarify the processes necessary for pediatric nurses to achieve practice that advocates children's rights. Findings revealed that the core category in this process is "being able to consider children centrally." This comprised of the following 4 grades, in the following order: 'One can act as instructed, but cannot consider things independently,' 'One obeys the tacit knowledge,' 'One can consider children centrally and can move forward,' and 'One carries out practice involving everyone, which takes into consideration the standpoint of the children.'

Further, three abilities were found to affect the intensity of this developed process: 'confirmation of children's power,' 'contrivance to convey children's power,' and 'attractive emotion to children.'
This article was published in the *Journal of Japanese Society of Child Health Nursing* (2016; 25(2): 8–15).

Parenting Stress Raising Infant Received Treatment of Retinoblastoma
The objectives of this study were to clarify characteristics of mothers' parenting stress and the relationships between the infants' illness-related factors, developmental characteristics, and parenting stress. A hospital-based study was conducted on 17 mothers of RB infants who were undergoing treatments to analyze longitudinal and cross-sectional data for both the treatment and follow-up period. The present study found that child-domain PSI scores were higher among mothers of bilateral RB infants with visual impairment than among mothers of infants without visual impairment, which tended to increase following the change from the treatment period to follow-up observation. Findings suggest that it may be helpful for nurses to refer mothers to developmental specialists for support if they feel concern or distress about their infant's development.
This article was published in the *Psychooncology (2016; 25: 1507–1511)*.

Reviews and Books

Nagayoshi M, Hirose T, Touju K, Suzuki S, Okamitsu M, Omori T, Kawamura A, Takeo N. Parenting Stress Related to Raising Infants Receiving Treatment for Retinoblastoma. *Psycho-Oncology.* 2016; **25:** 1507–11.

Maternity Nursing

Kimiko Kayashima, *Professor*
Yasuko Hososaka, *Associate Professor*

General Summary

Studies have been performed to examine the various health issues in each of the lifestyle stages of women and to explore how nursing assistance should be extended in maternal nursing.

Research Activities

The relationship between pregnancy behaviors of daily living/knowledge and the state of health guidance provided for pregnant women: with focus on first half of pregnancy
The objectives of this study are to examine the relationship between pregnant women pregnancy behaviors of daily living and knowledge in the first half of pregnancy and the status of healthcare guidance provided, and to explore the optimum approach to providing healthcare guidance during pregnancy. Survey questionnaires were distributed to 415

low-risk pregnant women in the first half of pregnancy,in the waiting room of the gynecology outpatients clinic. The five-point scale method was used to measure samples' responses to satisfaction factors concerning the last healthcare guidance, received, negative support, the level of knowledge before and after the healthcare guidance, and their pregnancy behaviors. This is a quantitative and descriptive study exploring correlation. The results, 273 of them from partnered primigravidae under 20 weeks gestation were used for analysis. The average gross pregnancy behavior score was 62.73 before healthcare guidance and 74.21 after healthcare guidance, showing a significantly higher result after healthcare guidance ($p<0.01$). Between the low satisfaction group 72.72 and high satisfaction group 75.62 concerning healthcare guidance, the high satisfaction group had a significantly higher pregnancy behavior score ($p<0.05$). These results indicate that the pregnance behavior score of pregnant women increases the higher the satisfaction of health guidance is following the start of health guidance, and the less negative support. The results indicate the importance of increasing communication skills during health guidance and conducting guidance under a receptive atmosphere.

Aspects of the boundary between discipline and abuse by mothers raising preschool age children

To analyze and clarify aspects of the boundary between discipline and abuse by mothers raising preschool age children based on analysis of narratives regarding parenting behaviors. Semi-structured interviews were conducted on 26 mothers raising preschool-age children focusing on the experiences they considered to reflect the boundary between discipline and abuse. Interview transcripts were qualitatively analyzed using a modified grounded theory approach. Categories reflecting aspects relating to the boundary between discipline and abuse were extracted as follows: "power to overwhelm the child unconsciously when their mother becomes emotional" and "differences in discipline depending on the attributes of the child". Additional categories included "superiority of other people's appraisal of discipline"; "accumulated fatigue from idealized images and responsibilities as a mother"; and "peace of mind to change according to surrounding support and mother's capabilities".

Community Health Nursing

Junko Shimasawa, *Professor*
Yoshiko Kubo, *Assistant Professor*
Yumiko Shimizu, *Assistant Professor*

General Summary

The faculty's research has been focused: 1) Visiting nursing care to promote continued community life by mentally ill patients living at home, 2) Development of the career anchors scale among occupational health nurses in Japan, 3) Relationship between career anchors and job and home life satisfaction among occupational health nurses in Japan, 4)

Health and welfare in hemodialysis patients who live in community, and 5) Nursing care for directly observed treatment short course.

Research Activities

Visiting nursing care for mentally ill patients living at home
The purpose of this study was to elucidate the features of assistance provided visiting nursing care to promote continued community life by individuals with mentally ill patients living at home. In this study, such assistance was considered to be support that promoted continued life in the community the mentally disabled individual in a manner suitable for that individual.

Development of the career anchors scale among occupational health nurses in Japan
This study aimed to develop the Career Anchors Scale among Occupational Health Nurses and to evaluate the reliability and validity. The variance contribution ratios of the 6 factors were 37.45, 7.01, 5.86, 4.95, 4.16, and 3.19%. The cumulative contribution ratio was 62.62%. Cronbach's alpha coefficient for the overall scale was 0.95, while those of the subscales were 0.88, 0.90, 0.91, 0.80, 0.85, and 0.79. The scale was found to be reliable and valid for measuring career anchors among OHNs in Japan.

Relationship between career anchors and job and home life satisfaction among occupational health nurses in Japan
This study examined the relationship between career anchors and job and home life satisfaction among Occupational Health Nurses (OHNs) in Japan.
The characteristics of career anchors among OHNs resemble previous studies. OHNs with more experience in their career have higher job satisfaction and we suggest that it is important for many OHNs to be appointed in managerial positions for career development.

Health and welfare in hemodialysis patients who live in community
This study is intended to clarify problems of health and welfare in hemodialysis patients who live in community. This year, we investigated pairs consisting of outpatients and their dialysis physicians in 118 dialysis facilities.

Nursing care for directly observed treatment short course
The purpose of this study was to elucidate the features of assistance provided nursing care to tuberculosis patient who received Directly Observed Treatment Short course in a hospital.

Publications

Kubo Y, Hatono Y[1], Kubo T[2], Shimamoto S[3], Nakatani J[1], Burgel BJ[5] ([1]Kyushu Univ) ([2]National Institute of Occupational Safety and Health) ([3]Tokai Univ) ([4]Univ of Occupational and Environmental Health) ([5]Univ of California, San Francisco). Development of the Career Anchors Scale among Occupational Health Nurses in Japan. *J Occup Health.* 2016; **58:** 519–

33.
Kubo Y, Hatono Y[1], Kubo T[2], Shimamoto S[3], Nakatani J[4], Burgel BJ[5] ([1]Kyushu Univ) ([2]National Institute of Occupational Safety and Health) ([3]Tokai Univ) ([4]University of Occupational and Environmental Health) ([5]University of California, San Francisco). Exploring Career Anchors among Occupational Health Nurses in Japan: A Qualitative Study. *Jpn J Nurs Sci.* 2016; **14:** 61–75.
Kubo T[1], Takahashi M[1], Liu X, Ikeda H[1], Togo F[2], Shimazu A[2], Tanaka K[3], Kamata N[3], Kubo Y, Uesugi J[4] ([1]National Institute of Occupational Safety and Health, [2]Tokyo Univ, [3]Kitasato Univ, [4]RIKEN, Institute of Physical and Chemical Research). Fatigue and sleep among employees with prospective increase in work time control: a 1-year observational study with objective assessment. *J Occup Environ Med.* 2016; **58:** 1066–72.
Sugisawa H[1], Shimizu Y, Kumagai T[2], Sugisaki H[3], Ohira S[4], Shinoda T[5] ([1]J.F. Oberlin Univ, [2]Osaka City Univ, [3]Hachioji Azumacho Clinic, [4]Sapporo Kita Clinic, [5]Kawakita General Hosp). Effects of socioeconomic status on physical and mental health of hemodialysis patients in Japan: differences by age, period, and cohort. *Int J Nephrol Renovasc Dis.* 2016; **9:** 171–82.

Home Care Nursing

Motoko Kita, *Professor*
Yuri Sugiyama, *Assistant Professor*
Hiroko Toyama, *Assistant Professor*

General Summary

Since 2011, our undergraduate course, Home Care Nursing, has focused on the acquisition of the ability to develop the nursing process based on the characteristics of home care nursing, in the process of studying home care nursing skills and home care nursing practice, which cover the theory to practical training. This year, we conducted an educational assessment of this course based on educational issues of each instructor.

Research Activities

Flipped Classroom teaching assessment in home nursing studies field; in an effort to retain knowledge

In order to teach the assessment viewpoint characteristic of home nursing, flipped teaching has been introduced where disease and symptom mechanisms that used to be taught in lectures are studied in advanced utilizing an e-learning system, and assessment points are taught intensively in lectures. The effect of flipped teaching was examined within the process so far, and the degree of understanding of assessment points didn't change depending on whether the prep-study lecture movies were watched or not, and the effectiveness of it could not be examined. So, a worksheet was introduced which allowed students to watch movies and organize the knowledge afterwards so they could retain those knowledge. As a result, it was proved that the students with higher self-evaluation saying they managed to organize new knowledge by using the worksheet, scored higher points in applicable area in regular exams. Further strategy that would lead to home nursing support theory is necessary utilizing the above in future.

A study of discharge support program construction for a case of older adult with dementia at an acute care hospital

In late years, increasing number of older adults with dementia are admitted to acute care hospitals to have other diseases treated, and discharge support for them are listed as difficult cases. A study using multiple case studies method is undertaken for the purpose of clarifying the discharge support process for older adults with dementia involving nurses of acute hospital's discharge support division, in order to develop a discharge support model corresponding to the difficulties characteristic of dementia.

A development of application for home visiting nurses, family caregivers and home visiting doctors to share information

At the scene of home treatment, it is extremely informant not only for the home visiting nurses and the doctors but also for the family members to share information to offer appropriate care at visits in order to assess them. Therefore, online application that can be used together by the 3 parties is being developed. In future, the application will actually be used and its usefulness will be tested.

Development of a Liaison Model for Pediatric Patients Using Multiple Home-visit Nursing Service Facilities

With an increase in the number of children with medical complexity living at home, the demand for home-visit nursing services is rising. However, at present, only limited numbers of facilities and nurses are capable of providing such services. As home-visit nursing service facilities tend to be small-scale, liaison among them may facilitate the strengthening of systems to support pediatric patients receiving home care and their families. Based on this, we are currently conducting a study to develop a liaison model for pediatric patients using multiple home-visit nursing service facilities.

Inspection/evaluation

Home Care Nursing has proactively introduced active learning into the class. Further class improvements must be made. We will continue our educational assessment in order to offer more effective education.

Since all research conducted by our instructors involves very important subjects in the field of home care nursing, we must support each other, and exert our utmost efforts to develop the course.

Publications

Kita M, Ito K[1], Ryu S[2] ([1]Univ Human Arts Sci, [2]Tokyo Women's Med Univ). Family Life Stability Scale for the Family Caring for Frail Elderly Persons. *Jikei Medical Journal.* 2016; **63:** 1-13.

Toyama H, Honda A[1] ([1]Tokyo Med Dental Univ). Using narrative approach for anticipatory grief among family caregivers at home. *Global Qualitative Nurs Res.* 2016; **3:** 1-15.

Index

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

V

W

X

Z

Research Activities 2016

The Jikei University School of Medicine

〔非売品〕

平成 29 年 12 月 1 日　発行

発 行 人　松 藤 千 弥

編 集 者　「教育・研究年報」編集委員長
南 沢　　享

英文研究年報担当（JMJ 編集委員長）
木 村 直 史

発　　行　東京慈恵会医科大学
〒 105-8461　東京都港区西新橋 3-25-8
電話　03-3433-1111

印 刷 所　笹氣出版印刷株式会社
〒 984-0011　仙台市若林区六丁の目西町 8-45
電話　022-288-5555

Published by
THE JIKEI UNIVERSITY SCHOOL OF MEDICINE
Tokyo, Japan