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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. Doctor Abe passed away on February 26, 2016. To him I dedicate this issue of *Research Activities*.

The Jikei University established the Centre for International Affairs in April 2015 to further promote its international activities. The University is sending more than 30 students abroad and receiving 65 overseas elective students in the academic year 2015. We have partnerships with 10 universities worldwide. A number of researchers are also doing joint studies in laboratories all over the world. I hope that *Research Activities* will promote the University's contribution to global society.

We owe much to the efforts of Professor Naofumi Kimura, Professor 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, 2016

Continuing Medical Education Center The Continuing Medical Education Committee

Hiroshi Tsuneoka, *Director* Yashuo 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

- Registered members: 200 (as of April 1, 2016) Members using the Center: 134/year Telephone service: 57 cases
- 2. The 36th summer seminar was held on August 1, 2015. 133 persons participated.
- 3. Monthly seminars were held on the second Saturday afternoons of the month in April, May, June, July, September, November, February, 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 bylaws of the Center were revised in 2013. 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 the Branch for Educational Institutional Research. 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, Dr. Okazaki and Professor Onoue engaged in implementation of new clinical practice program for fourth-years students starting in September.

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 project "Establish an Accreditation System for Basic Medical Education Compliant to Global Standards." In 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 making self-evaluation form as a first step in the accreditation process. Professor Fukushima joined external evaluation teams as chairs at Tsukuba University and Nara Medical University, as a vice-chair at Gifu University, and as a member at Yokohama City University. Assistant Professor Nakamura joined the external evaluation teams of Yokohama City University, Toyama University, and Gifu University in 2014 and 2015. In the activity, Prof. Nakamura visited King's College London to investigate data gathering, especially the Multiple Mini Interview, for entrance examinations.

3. Our proposal "Building of General Practice Capability from Undergraduate to Lifelong 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." The new program, medical care for the elderly program for third-year students, was implemented in March.

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 the planning and operation of a forum for medical education leaders (hosted by the Japan Medical Education Foundation) and a seminar for medical and dental education leaders (hosted by MEXT).

6. Professor Fukushima joined an expert committee (hosted by MEXT) revising the model core curriculum, and an action committee (hosted by the Tokyo Metropolitan Government) on community health in Tokyo.

7. Contribution to other institutions of higher education (faculty development lectures and workshops): Gifu University, Tokyo Medical and Dental University, IMS Group Patient Safety, Toyo Medical Practical School, Osaka Medical University, Iwate Medical University, Kyushu Dental College, Hyogo Medical School, Hyogo Medical School Hospital, YMCA-Yonago Welfare Practical School, Tohoku Bunka Gakuen University, South-Tama Nursing Practical School, Daito Bunka University, Sapporo Medical University, Hokkaido Pharmaceutical University, and Kumamoto University.

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 immunocytochemistry, electron microscopy, in situ hybridization histochemistry, single cell tracer injection and patchclamp electrophysiology. Our primary interest is focused on quantitative architecture and dynamics of microcircuit and their relationship. In gross anatomical researches, functional importance is explored on variations of organ systems using cadavers and animals.

Research Activities

Pattern differentiation of excitatory and inhibitory synaptic inputs on distinct neuronal types in the rat caudal nucleus of the tractus solitarius

Region- and size-specific neuronal organizations of the caudal nucleus of the tractus solitarius (cNTS) were investigated, followed by analyses of excitatory and inhibitory synaptic input patterns onto specific cell types by patch clamp recordings and immunoelectron microscopy. Cell size distribution and numerical density of cNTS neurons were examined in subregions at levels of the area postrema. In the subpostremal and dorsomedial subnuclei, characterized by the presence of dense glutamatergic and sparse GABAergic somata, small calbindin neurons constituted 42% of the total cells. The medial subnucleus contained large numbers of glutamatergic, GABAergic, and catecholaminergic somata and large tyrosine hydroxylase-containing cells constituted 13% in this region. In total, small neurons ($< 150 \text{ }\mu\text{m}^2$) represented about 80% of the cell population in the cNTS. Predominant excitatory postsynaptic currents were observed in the adult small neurons, while inhibitory postsynaptic currents were more evident in larger neurons, irrespective of subnuclear location. This distinct differentiation of postsynaptic current patterns was not evident in neonates. GABAergic synapses were more frequently associated with dendrites of large catecholaminergic cells (73%) than with those of small calbindin-containing cells (10%) in adults. These results indicate that differential synaptic input patterns were developmentally established in distinct small and large neurons.

Geometric and functional architecture of visceral sensory 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 visceral sensory microcircuit of the caudal nucleus of the tractus solitarius (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 is interested in the developmental and evolutional aspects of structure of the human body. By comparing organ development among vertebrates, we are attempting to reconstitute the evolutionary path that each of our organs has taken, at both the molecular and morphological levels, and, thus, are attempting to identify fundamental molecular mechanisms that shape each organ.

Research Activities

Histological analysis of the congenital ataxic mouse

Anterior horn cells in the spinal cord of the congenital ataxic mouse were examined with fluorescence Nissl staining. The L4 to L5 segments of the spinal cord were removed from congenital ataxic mice and phenotypically normal mice aged 4, 10, and 20 weeks. The spinal cord segments were serially sliced at a thickness of 150 μ m and stained with an aqueous solution of cresyl violet. The sections were mounted with a polyester resin and observed throughout the whole thickness with a laser scanning microscope. The number of anterior horn cells in ataxic mice was comparable to that in phenotypically normal mice. No degenerative changes were found in the anterior horn cell of either ataxic or normal mice. These results indicate that the peripheral motor neuron is not affected in the congenital ataxic mouse.

Primitive actinopterygian Polypterus has superficial neuromasts in the lateral line

Polypterus, the most basal extant actinopterygian fish in molecular phylogeny, possesses ganoid (enamel) scales on the surface of the body, which reminds us of an extinct primitive actinopterygii or teleostei, such as *Psarolepis* or *Lophosteus*. 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 mechanosensory or electrosensory 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 represent-ative model of a primitive actinopterygian.

Initial neuromast cells appeared in neurula as the placode and the neural crest cells to coalesce into apparent rosette structures in bistratal epidermis, which was observed 5 days after fertilization. At the hatching larval stage, the lateral line neuromasts, which bulged

out from the surface of the epidermides, projected their axons inward toward the lateral line nerve. The neuromast will finally be buried in the adult under the superficial epidermis with a small pore in the surface, which was reported last year.

Therefore, the lateral line neuromasts along the body existed superficially for life and did not form a distinct canal organ, even after mineralization; this finding indicates that the lateral line neuromast in the trunk of *Polypterus* is a superficial organ. Moreover, the development pattern of the neuromast in *Polypterus* resembles that in the zebrafish, as previously reported.

Transcriptome analysis of the developing diaphragm

Congenital diaphragmatic hernia (CDH) caused by a defect of diaphragmatic development. However, the pathogenic mechanism of CDH is still unknown. Because the diaphragm consists of several tissues from around its area of development, understanding the cells' origin and location in the diaphragm can be difficult. Therefore, determining which tissues comprise which part of the diaphragm is important to understand diaphragmatic development. To investigate this issue, we performed several studies and found that some tissues were asymmetrically distributed in the diaphragm. We hypothesized that this asymmetrical distribution of some tissues contributes to the occurrence of CDH. This year, we attempted a more detailed analysis of gene expression to understand these asymmetrical tissues. We divided the developing diaphragm into 6 parts, in each of which a transcriptome was constructed. From the gene expression patterns of each part, we found that some genes were expressed only on the right side and are well known to be responsible for CDH. We believe that these asymmetrical tissues indicate that different genes are expressed in left-right asymmetry and might contribute to the occurrence of CDH. Our future studies will investigate the pathogenic mechanism of CDH.

Region-specific heparan sulfate proteoglycan function in the zebrafish fin development

Proteoglycans, which are proteins with glycosaminoglycan chains, act as the extracellular matrix responding to the biochemical and mechanical cues. Heparan sulfate proteoglycans (HSPGs) are synthesized by exostosin genes (EXT), which encode glycosyltransferases. The protein exostosin-like glycosyltransferase 3 (EXTL3) is an N-acetylglucosamine transferase in the initial step of HSPG synthesis, and in embryogenesis, EXTL3 gene is expressed ubiquitously. Interestingly, extl3-mutant zebrafish do not form the distal part of the pectoral fin in development. To investigate functions of other ext genes in pectoral fin development, we have generated gene knockout fish with the CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats-CRISPR-associated protein 9) system. We have found some malformations (abnormal chondrogenesis and loss of the distal part of the fin) of the pectoral fin primordia with double knockout of the genes for ext1b and ext1c. Previous studies suggest that ext2 is required for the proximal part of the fin, which is homologous to limbs of tetrapods. Our data suggest that the genes *ext1b*, extlc, and extl3 are required for the distal part of the fin in development and that the fish-specific structure, which is called the fin-ray, is formed by the region-specific HSPG function.

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Department of Molecular Physiology

Shigeru Takemori, Professor Toshiko Yamazawa, Assistant Professor Maki Yamaguchi, 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

X-ray diffraction study of extraocular muscle

To explore the structural bases of the unique function of extraocular muscle at the molecular level, an X-ray diffraction experiment was performed. First, we measured 1,0 lattice spacing of extraocular muscle fibers in the function of sarcomere length, which is the most representative variable of myofilament structure. The 1,0 lattice spacing of extraocular muscle fibers in distal and end plate regions was found to exhibit the same relationship with psoas fibers. The relationships did not differ from each other for the subgroup consisting of orbital and global layers and indicate that extraocular muscle fibers have the same sarcomere structure from the end plate to the distal region in both layers.

Next, we observed profiles of equatorial reflections of extraocular muscle fibers which give information about the mass distribution of muscle proteins arising from the 1,0 and 1,1 lattice planes. Equatorial reflections of extraocular muscle fibers showed patterns essentially similar to those of psoas fibers, as suggested with electron microscopic observation. However, equatorial reflections, especially 1,0 reflection, appeared to be wider in extraocular fibers than in psoas fibers. Also, within the results from extraocular fibers, 1,1 reflection in samples from the end plate region tended to be lower than that from the distal region. Averaged (1,1/1,0) intensity ratios of equatorial reflections of each species of muscle fiber specimens which have statistically the same sarcomere length distribution showed that the (1,1/1,0) intensity ratio was lower in extraocular muscle fibers than in psoas muscle fibers, suggesting that different kinetic properties of myosin may be a cause of the unique function of extraocular muscle.

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 by absorbing extra heat with temperature similarly to the melting of ice. With differential scanning calorimetry, we observed the absorption of extra heat with the temperature on skinned fibers. We observed 2 significant extra heat absorptions at -22° C, -25° C, and at about the melting point of water. Additionally, we observed 2 more peaks at 45° C and 65° C in a temperature-dependent irreversible manner. The 2 peaks at less than -10° C were affected by the denaturation and the presence of thick/thin filaments. Furthermore, the effect of the presence of thick filaments was different from that of the presence of thin filaments. We found that specimens with intact thin filaments had a larger specific heat capacity (accumulated heat from -80° C to $+20^{\circ}$ C). These results suggest that 1) differential scanning calorimetry can be used to effectively explore the interaction between myoproteins and water and that 2) thin filaments serve a special heat capacity.

Property of water around myoprotein studied with quartz crystal microbalance and nuclear magnetic resonance

We observed the adsorption process of myosin to the gold surface by quartz crystal microbalance to study viscoelastic property of the myosin and its surrounding solution as a whole by means of a quartz crystal microbalance molecular interaction analyzer (AFFI-NIXQN Pro, Initium, Inc., Tokyo).

When myosin adsorption was sparser than 0.2 μ g/cm², viscoelastic change accompanied by myosin adsorption was almost the same as 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 head domain of myosin changes the viscoelasticity of the protein. This change suggests that myosins immobilize a surrounding solution when it is closely adsorbed. Half of this immobilized solution was released in the presence of ATP or ADP but not in the presence of ATP- γ S.

We also observed the spin-spin relaxation process of ¹H nuclear magnetic resonance signals with the suspension of myosin filaments of rabbit psoas muscle in the 4 major intermediates during the ATP-hydrolysis by myosin. The results imply that myosins in the M and M.T states immobilize many water molecules and that myosins in the M.D.Pi and M.D states release water molecules.

Exocrine glands observed in aqueous solution with atmospheric scanning electron microscopy

Exocrine glands, e.g., salivary and pancreatic glands, play an important role in digestive enzyme secretion, while endocrine glands, e.g., pancreatic islets, secrete hormones that regulate blood glucose levels. The dysfunction of these secretory organs immediately leads to various diseases, such as diabetes and Sjögren's syndrome, by poorly understood mechanisms. Glands and gland-related diseases have been studied by optical microscopy, and at higher resolution by transmission electron microscopy of Epon-embedded samples, which necessitates hydrophobic sample pretreatment. We challenged the direct observation of tissue in aqueous solution by atmospheric scanning electron microscopy (SEM).

Salivary sublingual glands, lacrimal glands, and pancreas were fixed, sectioned into slabs, stained with phosphotungstic acid, and inspected in radical scavenger D-glucose solution from below with inverted SEM, guided by optical microscopy from above to help target the tissue substructures. A specimen thickness of 2 to 3 μ m was visualized with SEM. In secretory cells, cytoplasmic vesicles and other organelles were clearly observed at high resolution, and the cytoplasmic vesicles could be classified according to the degree of phosphotungstic acid staining. In the islets of Langerhans, the microvascular system used as an outlet by the secretory cells was also clearly observed. The microvascular system is also critically involved in the onset of diabetic complications and was clearly visible in subcutaneous tissue observed with atmospheric SEM. The results suggest that in-solution atmospheric SEM can be used for histology and to study vesicle secretion systems. Furthermore, the high-throughput of atmospheric SEM makes it a potential tool for diagnosing exocrine-related diseases, such as Sjögren's syndrome.

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Department of Cell Physiology

Susumu Minamisawa, Professor Norio Fukuda, Associate Professor Masato Konishi, Visiting Professor Yoichiro Kusakari, Assistant 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 the pulmonary vein. In addition, we developed an *in vivo* nanoimaging system to observe sarcomere contraction in the ventricles of small animals, such as rats and mice.

Research Activities

Development and pathogenesis of the great arteries

1. Molecular mechanism of closure of the DA

The DA is a mysterious artery that attracts considerable research interest. The DA is an essential artery that connects the main pulmonary artery and the descending aorta in a 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.

Decreased elastogenesis is a hallmark of structural change in the DA and is thought to contribute to intimal thickening of the DA. However, the molecular mechanisms of decreased elastogenesis are not fully understood. We found that signaling of the prostaglandin E receptor EP4 promotes degradation of the mature lysyl oxidase protein that is a cross-linking enzyme for elastic fibers in the DA, leading to decreased elastogenesis. Both the avian DA and the mammalian DA close after birth. Although the avian DA has characteristics of vascular structure that differ from those of the mammalian DA, the poor elastogenesis is similar. We are examining the chicken DA to elucidate the molecular mechanisms by which elastogenesis is impaired in this specific artery.

2. Causal factors of aortic coarctation

Aortic coarctation is a congenital heart disease in which the descending aorta is narrow, usually in the area where the DA connects. In some cases, re-narrowing of the aorta develops after definitive operation. We found that DA smooth muscle cells were straying into aortic smooth muscle cells of the narrowed area. We are now elucidating the molecular mechanisms of aortic coarctation. We are collaborating in this study with Hyogo Prefectural Kobe Children's Hospital.

Regulation of cardiac sarcoplasmic reticulum ATPase activity

Impaired Ca^{2+} reuptake into the sarcoplasmic reticulum is thought to be a primary pathogenic mechanism of heart failure. We are interested in regulation of the sarcoplasmic reticulum Ca^{2+} -ATPase and Ca^{2+} homeostasis in the sarcoplasmic reticulum. We generated sarcolipin-Cre knockin mice in which Cre recombinase is inserted in the sarcolipincoding region. Although sarcolipin homozygous deletion exhibited an increase in Ca^{2+} -ATPase activity in the atria, heterozygous deletion of sarcolipin generally had no phenotype. Therefore, sarcolipin-Cre knockin mice could be used to generate an atriumspecific gene deletion mouse, because sarcolipin is specifically expressed in the mouse atria.

Regulation of cardiac metabolism

Cardiac metabolism plays an essential role in maintaining cardiac function. The energy of cardiac muscle largely depends on fatty acid oxidation. The main cardiac metabolism is known to switch from fatty acid oxidation to glycolysis when the heart is exposed to stress. Vitamin B1 (thiamine) deficiency causes beriberi, which is characterized by peripheral sensory and motor neuropathy and congestive heart failure. Dr. Kanehiro Takaki, the founder of The Jikei University, eliminated beriberi from the Imperial Japanese Navy by improving its food supply (thiamine supplementation). We hypothesized that vitamin B1 derivative products (thiamine pyrophosphate) would protect the heart against ischemia/reperfusion injury. We found that pretreatment with vitamin B1 preserves cardiac function in cases of ischemia/reperfusion injury. We are now investigating the mechanism of these effects.

Pathophysiological mechanisms of cardiac remodeling and fibrosis

Cardiac fibrosis is a maladaptive response to pathophysiological conditions, such as in cardiac hypertrophy and ischemic heart diseases. However, the effects of interstitial fibrosis on Ca^{2+} handling and contraction in myocardium remain unclear. We prepared pulmonary artery banding (PAB) rats as a model of cardiac hypertrophy. Four weeks after the operation, the right ventricular papillary muscles of the PAB rats were dissected and their tension was measured with intracellular Ca^{2+} transients by means of the photoprotein aequorin. On the basis of histological analysis, papillary muscles after PAB were clearly divided into 2 groups: the interstitial fibrosis group and the nonfibrosis with hypertrophy group. Using DNA microarray analyses, we found that fibroblast growth factor 23, which is known to play a role in the regulation of osteogenesis, was up-regulated in the interstitial fibrosis group. We are now investigating the role of fibroblast growth factor 23 in the development of cardiac fibrosis.

Mechanism of sarcomere contraction in cardiac muscle

1. Sarcomere length nanometry in rat neonatal cardiomyocytes via expression of α -actinin-*Aequorea coerulescens* green fluorescent protein in Z-disks

In cardiac muscle, a change in sarcomere length by a mere 100 nm causes a dramatic change in contractility, indicating the need for the simultaneous measurement of sarcomere length and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in cardiomyocytes at high spa-

tial and temporal resolutions. To accurately analyze the motion of individual sarcomeres with nanometer precision during excitation-contraction coupling, we applied nanometry techniques to primary-cultured rat neonatal cardiomyocytes. We developed an experimental system for simultaneous nanoscale analysis of single sarcomere dynamics and $[Ca^{2+}]_i$ changes via the expression of *Aequorea coerulescens* green fluorescent protein in Z-discs. We found that the averaging of the lengths of sarcomeres along the myocyte, a method generally now used in myocardial research, caused the sarcomere lengthening speed to be markedly underestimated owing to the superposition of different timings for lengthening between sequentially connected sarcomeres. The present experimental system has a broad range of possible applications for unveiling single sarcomere dynamics during excitation-contraction coupling in cardiomyocytes under various settings.

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*. We have developed a high-speed high-resolution *in vivo* cardiac imaging system in mice. This system enables 3-dimensional analysis of sarcomere dynamics during the cardiac cycle, simultaneously with electrocardiography and left ventricular pressure measurements. We demonstrated that the working range of sarcomere length exists on the shorter resting distribution side and that the developed pressure is a linear function of the sarcomere length change between diastole and systole at 100-nm levels.

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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

The identification of effective treatment for breast cancer with low expression of dual specificity tyrosine phosphorylation-regulated kinase 2

Our recent study revealed that dual specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2) has a tumor-suppressive function through the expression of c-Myc, c-Jun, and Snail and the phosphorylation of p53. The expression of DYRK2 is decreased in advanced breast cancer and serous ovarian cancer. The decreased expression of DYRK2 causes breast cancer to be resistant to cytotoxic chemotherapy and to have a poorer prognosis. However, a therapeutic strategy has not been established for breast cancers with low DYRK2 expression. The mechanistic target of rapamycin (mTOR) complex 1 pathway was shown with microarray analysis to be activated in DYRK2-depleted cells. Treatment with everolimus, an inhibitor of mTOR, was associated with a significant inhibition of tumor growth compared with a vehicle *in vitro* and *in vivo*.

This year, we found that DYRK2 phosphorylates mTOR at 631T to degrade mTOR through the ubiquitin-proteasome pathway by F-box and WD repeat domain-containing protein 7. Moreover, patients with low DYRK2 expression required a longer treatment period and had a higher clinical benefit rate than did patients with high DYRK2 expression.

Elucidation of the regulatory mechanism of breast cancer stem cells

Cancer stem cells have tumorigenic potential. Breast cancer stem cells are detected by CD44 high/CD24 low. In DYRK2-depleted cells, the expression of Krüppel-like factor 4 (KLF4) was upregulated and resulted in an increased population of the cancer stem cell. This year, we analyzed the clinical samples. Low DYRK2 expression was significantly correlated with an upregulation of CD44⁺/CD24⁻ and aldehyde dehydrogenase 1-positive cancer stem cell population. We identified androgen receptor as a transcription factor,

binding to the KLF4 promoter region, which is dependent on the activity of DYRK2 kinase. Our findings provide a mechanism of cancer stem cell regulation through the DYRK2-androgen receptor-KLF4 axis in breast cancer. This pathway is thus a potential therapeutic target for the breast cancer stem cell.

Cancer-associated thyroid hormone receptor interacting protein 13

Thyroid hormone receptor interacting protein 13 (TRIP13) is the mammalian ortholog of pachytene checkpoint protein 2 (Pch2), which in yeast regulates several meiotic processes, such as synaptonemal complex formation, interhomologous recombination, and the repair of DNA double-strand breaks. A recent report suggested that TRIP13 promotes error-prone nonhomologous end-joining and induces chemoresistance in head and neck cancer. We attempted to determine TRIP13 subcellular localization under physiological and DNA-damaged conditions using immunofluorescence techniques. We found that TRIP13 was located in cytoplasm and that its distribution did not change after DNA damage. The expression profile of TRIP13 is highly restricted and most abundant in the testis but is aberrantly expressed in multiple types of cancer. Nevertheless, the functional roles of TRIP13 in cancer-cell growth and survival are largely unknown. Through the short hairpin RNA expression system, long-term knockdown of TRIP13 resulted in significant arrests of cell growth in several human cancer cell lines. In particular, in the breast cancer cell line MCF7, TRIP13 knockdown exhibited cell senescence-like phenotypes, such as cytoplasmic enlargement and multinucleated cell formation. Additionally, TRIP13knockdown MCF7 cells had enhanced expression of p21 (WAF1/CIP1) and phosphorylation of AMP kinase. Transcriptional regulation of TRIP13 expression was not investigated. We characterized the promoter region of the TRIP13 gene (TRIP13) by bioluminescence-based reporter assay. A 2.7-kb upstream fragment from the initiation codon of TRIP13 and its deletion series were cloned into a luciferase reporter vector. Maximum levels of luciferase activity were detected with the 369-bp fragment from the initiation codon.

Polo-like kinase 1 regulates mitotic chromosome condensation

Chromosomal aberration and genomic instability are hallmarks of cancer. A large proportion of cancer cells is aneuploid and contains incorrect numbers of chromosomes. We focused on Polo-like kinase 1 (Plk1), an essential regulator for proper mitotic progressions which is overexpressed in several cancers. To investigate Plk1 functions in mitosis, aneuploid cancer cell lines were treated with a Plk1 inhibitor. We found that inhibition of Plk1 leads to a striking reduction during mitosis of chromosome-associated protein (CAP)-H2, which is a subunit of condensin II that contributes to mitotic chromosome condensation and segregation. We performed further analysis and found that inhibition of Plk1 leads to anaphase-promoting complex/cell division cycle protein 20 (APC/Cdc20)mediated degradation of CAP-H2 in mitosis. We also demonstrated that Plk1 phosphorylation of CAP-H2 at Ser288 is required for the stabilization of CAP-H2 and accurate chromosomal condensation during mitosis. These findings suggest that Plk1-mediated phosphorylation controls condensin II functions by modulating CAP-H2 expression levels to facilitate proper mitotic chromosome condensation.

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Department of Molecular Biology

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

General Summary

Polyamines (putrescine, spermidine, and spermine) are ubiquitous biogenic amines that bind mainly to nucleic acids and are essential for cell proliferation. Ornithine decarboxylase (ODC) is a key enzyme of polyamine biosynthesis in mammalian cells. ODC converts ornithine to putrescine, which in turn leads to spermidine and spermine. ODC is degraded by interaction with antizyme (AZ). Three AZ isoforms (AZ1-3) are present in mammals. The AZs are expressed by translational frameshifting that is induced by polyamines and negatively regulate cellular polyamines. Cellular polyamine contents are maintained by the feedback mechanism involving AZ. The AZs are further regulated by proteins termed AZ inhibitors (Azins). Cancer cells generally contain elevated levels of polyamines. Our goal is to clarify the mechanism and biological significance of the elaborate regulatory system and to develop polyamine-related research or diagnostic tools.

Research Activities

Interaction between MYCN and AZ2 in neuroblastoma cells

We have found that AZ2 interacts with the cellular myelocytomatosis oncogene protein (c-MYC) in the nucleolus and nucleoli and accelerates c-MYC degradation in a ubiquitin-independent manner. This year we tested interaction between AZ2 and another member of the v-myc avian myelocytomatosis viral oncogene homolog (MYC) family, the MYC neuroblastoma derived homolog (MYCN), which is overexpressed in neuroblastoma cells. The high-level expression of AZ2 has been reported to correlate with increased survival in patients with neuroblastoma. Pull-down assay demonstrated that AZ2 specifically interacted with MYCN in a human neuroblastoma cell line, SH-SY5Y. In addition, binding of AZ2 accelerated MYCN degradation. This result suggests that AZ2 accelerates MYCN degradation in an ubiquitin-independent manner as it accelerates ODC or c-MYC degradation.

Analysis of interaction between AZ2 and ATP citrate lyase

Previous screening for AZ-binding proteins identified ATP citrate lyase (ACLY), a cytosolic enzyme that catalyzes the production of acetyl-CoA. We confirmed that both AZ1 and AZ2 bind to ACLY and that AZ colocalizes with ACLY to the cytoplasm. Unexpectedly, neither AZ1 nor AZ2 accelerated ACLY degradation. Instead, purified AZ, particularly AZ1, increased the activity of purified ACLY in a dose-dependent manner *in vitro*, suggesting that AZ activates ACLY through protein-protein interaction. Polyamines had no effect on ACLY activity *in vitro*. Knockdown of AZ1 or AZ2 or both in human cancer cells significantly decreased ACLY activity and the cellular levels of acetyl-CoA and cholesterol, indicating that AZ regulates acetyl-CoA production in living cells.

Heterogeneity of hematopoietic stem cells in AZ1 knockout mice

We continued our research of AZI knockout $(AZI^{-/-})$ mice. The significant features of $AZI^{-/-}$ mice are the increased putrescine level in tissues and the partial embryonic lethality with severe anemia. Last year, we showed the heterogeneity of hematopoietic stem cells (HSCs) from fetal liver, which have long-term bone marrow reconstruction ability. To study whether HSCs, which migrate from fetal liver to bone marrow in the late embryonic stage, have heterogeneity that continues for life, we collected $AZI^{-/-}$ bone marrow cells from 6-month-old mice, transplanted the bone marrow cells including the HSCs into recipient mice, and assayed the repopulating potential of the HSCs. The $AZI^{-/-}$ HSCs from the bone marrow indicated heterogeneity similar to that of HSCs from the fetal liver.

Analyses of physiological roles of Azin1

To address the regulatory roles of Azin1, we used spontaneously immortalized mouse embryonic fibroblasts (MEFs) from wild-type mice and mutant mice, in which expression of Azin1 is greatly decreased. We analyzed metabolite profiling of MEFs with capillary electrophoresis-mass spectrometry. Strong metabolic abnormalities in polyamines, folate, and nucleotide metabolism were noted in the mutant MEFs. Next, cell cycle progressions of MEFs were analyzed with flow cytometry using propidium iodide. The G2M ratio was found to be increased. The sub-G1 (hypodiploid) peak, which was regarded as specific for apoptosis, was noted in the mutant MEFs.

Analysis of AZ frameshifting mechanism with human in vitro translation systems

Using human *in vitro* translation systems, we are analyzing the molecular mechanism of ribosomal frameshifting of AZ expression. Polyamines induce both ribosomal frameshifting and read-through at stop codons. As a result, multiple translation products are detected in these systems, and the exact frameshift efficiency is difficult to analyze. To prevent this problem, the reporter for these systems was improved to synthesize single frameshift products and single read-through products. The stop codons in read-through frames were replaced with codons for alanine. In addition, the read-through product was translated as a green fluorescent protein fusion protein to be separated from the frameshift product on sodium dodecylsulfate-polyacrylamide gel electrophoresis. All synthesized peptides are released by the virus peptide release signal independent of the stop codon. This reporter expressed expected products in both the HeLa cell extract system and the human Protein synthesis Using Recombinant Elements (PURE) system. We are performing experiments with this reporter to examine the molecular mechanism of AZ frameshifting.

Interaction analysis of spermine-binding RNA aptamers with spermine

Aptamers of RNA are useful for exploring RNA-binding sequences and structures for target molecules. The spermine-binding aptamer SL_2 consists of 2 stems: the terminal-side stem and the loop-side stem separated by an internal loop. These structures were necessary for their spermine-binding activities. Replacement of an A-U base pair adjacent to an internal loop in the terminal-side stem with a G-C base pair (SL_2 GC) reduced spermine-binding activity. Nuclear magnetic resonance signals of the terminal-side stem in SL_2 were observed to be broader than signals in SL_2 GC, indicating that the terminal-side stem of SL_2 takes a loose structure. We propose that the loose stem provides the plasticity to the RNA and is necessary for the spermine-RNA interaction.

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Department of Pharmacology

Toshihiko Momiyama, Professor Yuji Ohno, Assistant Professor Taro Ishikawa, 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. Study of the mast cell allergic response (Haruhisa Nishi)

5. Analysis of the cerebrocerebellar interaction using optogenetics (Taro Ishikawa, Misa Shimuta)

6. The basic mechanism underlying anticonvulsant effects of a ketogenic diet (Masahito Kawamura)

7. Coupling distance between presynaptic Ca²⁺ 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 local neural circuits.

These basic analyses could 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

Aquatic turtles, amphibians, and air-breathing fishes, unlike aves and postnatal mammals, never open their glottis while yawning. Elasmobranches that lack lungs and a glottis also show yawn-like behavior. Therefore, the central pattern generator of yawns is considered phylogenetically older than that of pulmonary ventilation. Coupling between lung-ventilation and yawning might have evolved as a result of the change into air-aspirating

breathing.

Peripheral benzodiazepine receptors on adrenal cells

Peripheral benzodiazepine receptors localize in the outer mitochondrial membrane; they transfer cholesterol in steroidogenic organs under physiological conditions and are 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, are related to these pathological conditions.

Study of the mast cell allergic response

An increase of the intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) is recognized as being essential for mast cell activation, leading to the release of histamine. However, the release of the histamine dependencies of the different pathways leading to the rise in intracellular Ca^{2+} in activated mast cells is unknown. To investigate this association, the relationship between the $[Ca^{2+}]i$ increase and the release of histamine caused by variable stimulation, was examined in LAD2 cells, a human mast cell line that can be activated to the release of histamine. The results indicate that extracellular Ca^{2+} influx is more critical to release of histamine than to Ca^{2+} mobilization from the intracellular organelles in LAD2. These results suggest that controlling the Ca^{2+} influx pathways could be a useful way to mitigate the allergic symptoms caused by activated mast cells.

Analysis of cerebrocerebellar interaction by means of optogenetics

The circuit that connects the cerebrum and the cerebellum is important in a wide range of brain functions, including sensory information processing. We are investigating the neural circuits involved in the orofacial sensory signal processing of mice. We found that, among the signals recorded in the cerebellar granule cells, signals that had a long latency were specifically suppressed by cerebral optogenetic inhibition. In contrast, signals that had a short latency, which originated from the trigeminocerebellar pathway, were not suppressed. These results suggest that direct trigeminal signals and indirect cerebral signals are integrated in cerebellar granule cells.

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 animals fed a ketogenic diet showed reduced excitability, and the effects of the ketogenic diet could be reversed with blockers of adenosine A1 receptors. These results suggest that the reduction of neuronal activity through activation of adenosine A1 receptor is a key mechanism underlying the anticonvulsant effects of a ketogenic diet.

Coupling distance between presynaptic Ca²⁺ *channels and synaptic vesicles*

The coupling distance between voltage-gated Ca2+ channels and synaptic vesicles criti-

cally determines the probability and timing of neurotransmitter release. The presynaptic terminal of the calyx of Held in the auditory brainstem is composed of stalks connecting to the axon and of swellings extended from stalks, both of which form release sites onto the postsynaptic neuron. Electrophysiological measurements combined with numerical simulations of transmitter release at this synapse revealed that the coupling distance is longer in swellings than in stalks. This result suggests that the coupling distance between Ca^{2+} channels and vesicles varies among release sites within the same synapse, thereby diversifying the method of transmitter release.

Cholinergic modulation of central synaptic transmission

Acetylcholine is a neurotransmitter involved in learning and memory. In the central nervous system, several studies have shown that synaptic transmission and the firing property of neurons are modulated by acetylcholine. We examined cholinergic modulation in the striatum and the hippocampus with an electrophysiological technique. In the striatum, we have found that the GABAergic inhibitory transmission onto cholinergic interneurons is inhibited by the activation of muscarine receptors.

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Department of Pathology

Masahiro Ikegami, Professor Masafumi Suzuki, Professor Satoru Chiba, Associate Professor Kohichi Nomura, Associate Professor Yasuhiko Endo, Assistant Professor Tohru Harada, Assistant Professor Masakazu Komine, Assistant Professor Akihiko Sakata, Professor Takako Kiyokawa, Professor Hiroyuki Takahashi, Associate Professor Yukiko Kanetsuna, Assistant Professor Shigeharu Hamatani, Assistant Professor Tomoe Lu, 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 with light microscopy, electron microscopy, morphological measurement, immunohistochemical staining, and molecular pathological techniques.

Research Activities

Research on the gastrointestinal tract

1. Budding, lymphatic invasion, and venous invasion are known risk factors for lymphnode metastasis in cases of colorectal cancer with submucosal invasion. We studied the relations between combinations of budding, lymphatic invasion, and venous invasion and the presence or absence of lymph-node metastasis in 22 endoscopically resected submucosal cancers for which the presence or absence of lymph-node metastasis was determined (Katsushika Medical Center, from January 2012 through October 2015). Lymphnode metastasis was present in 2 of the 22 lesions. The lymph-node metastasis was associated with the combinations of budding + lymphatic invasion and of budding + lymphatic invasion + venous invasion.

2. To evaluate the histopathological characteristics of villous adenomas, 74 lesions with villous component in part of the adenoma were identified by reviewing colorectal epithelial tumors that were stored at Katsushika Medical Center (2009-2016). Regions of tubular component were found in all of these lesions. Regions of villous component, tubular component, and normal glands were manually resected from 1 lesion with villous capillaries, and the presence or absence of mutations of the Guanin nucleotide binding protein, alpha stimulating gene (GNAS) complex locus gene was compared. The results showed that *GNAS* mutations can be found in regions of villous component, whereas no mutations were found in regions of tubular component.

Research on female genital organs

1. In patients with ovarian clear-cell carcinoma associated with underlying endometriosis, AT-rich interactive domain 1A gene (ARID1) abnormality might be related to both carcinogenesis and tumor progression. Interleukin 6 provides control signals for *ARID-1*, but

no correlation was found between ARID-1 abnormalities and interleukin 6 expression in ovarian clear-cell carcinoma.

2. Recent studies have shown that high-grade serous carcinoma (HGSC), usually considered a primary tumor arising in the ovaries or peritoneum, can be due to the metastasis of fallopian tube cancer to the ovary or peritoneum and that the organ with the largest mass is not necessarily the site of the primary tumor. In patients with a clinical diagnosis of bilateral ovarian cancer, detailed examination of surgical specimens of the fimbriae of the fallopian tube showed HGSC and contiguous intraepithelial serous adenocarcinoma in the fimbriae of the fallopian tube. Primary cancer of the fimbriae of the fallopian tube was diagnosed. In cases of HGSC, the primary lesion can be identified with detailed observation and examination of the fimbriae of the fallopian tube.

Research on urogenital organs

1. Primary lesions and metastatic lesions of urothelial cancer were immunostained (human epidermal growth factor receptor [HER2], p53, and Ki-67), and HER2 was evaluated with the use of immunohistochemical staining according to the guidelines of the American Society of Clinical Oncology and the College of American Pathologists. In patients with 2+ immunohistochemical staining, fluorescence in-situ hybridization was performed to assess gene amplification. The results showed that 3+ staining was present in 17% of cases. Gene amplification was found with fluorescence in-situ hybridization in 6 cases with 2+ staining. The rate of positivity for HER2 did not differ significantly between patients with metastasis and those without metastasis.

2. Continuing from the previous year, we studied the clinical histopathological characteristics of prostate cancers of the anterior and transition zone and those of the posterior and marginal zone. Immunostaining for phosphatase and tensin homologue (PTEN) and serine peptidase inhibitor, Kazal type 1 (SPINK1) was performed to compare expression levels in each zone. In addition, *SPINK1* expression was compared between cancers that were positive or negative for erythroblast transformalion specific related gene (ERG). In prostate cancers of the anterior and transition zone, the incidence of *PTEN* loss was significantly lower than that in prostate cancers of the posterior and marginal zone. The expression of *SPINK1* did not differ significantly. The expression of *ERG* and that of *SPINK1* were mutually exclusive.

Research on the liver

1. In patients with primary biliary cirrhosis who underwent several biopsies, histological changes were assessed according to Nakanuma's classification to investigate whether such changes correlate with changes in biochemical data. A correlation between clinical findings (changes in biochemical data) and pathological findings was often seen in patients in whom changes in pathological findings were inconsistent with clinical data. Histological evidence of inflammation and chronic nonsuppurative destructive cholangitis were seen in some patients despite improvements in such variables as alkaline phosphatase and γ -glutamyl transpeptidase. During the follow-up of patients with primary biliary cirrhosis both blood chemical testing and liver biopsy should be performed if possible.

Research on the mammary gland

1. Data on approximately 300 cases of benign and borderline lesions were collected and computerized. The pathological diagnosis of some intraductal lesions varied from benign (hyperplasia) to malignant (noninvasive ductal carcinoma), depending on the pathologist. On immunostaining for actin, p63, and CD10, positive staining at intratubular sites of hyperplasia (distinctly biphasic) was associated with benign papilloma. However, positive staining only around the ducts (myoepithelium present) was associated with great variation in the lesion being diagnosed as benign or malignant.

Molecular pathological research

1. We investigated new responsible genes related to the development and progression of primary lung cancer. To discover the locations of these genes, microsatellite instability (MSI) analysis was performed with the polymerase chain reaction and 19 DNA markers at chromosome 8p to evaluate a total of 306 cases of lung adenocarcinoma, squamous-cell carcinoma, or neuroendocrine tumor. The incidence of MSI was found to be 20% at 8p23.2, 51% at 8p23.1, 24% at 8p22, and 15% at 8p21. The incidence of MSI was significantly higher at 8p23.1 than at other regions. In particular, the incidence of MSI at DNA marker *D8S1819* was high in each histologic type of lung cancer, suggesting that a responsible gene related to the development of lung cancer is present at 8p23.1.

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Department of Virology

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Nobuyuki Kobayashi, Assistant Professor

General Summary

Human herpesviruses (HHVs) are capable of establishing lifelong latent infections of their hosts and are frequently reactivated. We are studying the molecular mechanism of latency and pathogenesis of human cytomegalovirus (HCMV) and HHV-6, and have found a novel latent protein of HHV-6 associated with chronic fatigue syndrome and mood disorders. Additionally, we are attempting to apply HHV-6 and HHV-7 as tools to study the mechanism of fatigue. The amounts of salivary HHV-6 and HHV-7 DNA increased with training and decreased with rest, suggesting the usefulness of these types of DNA as biomarkers of physiological fatigue. Additionally, we study cognitive impairment and Alzheimer's disease (AD), which we have previously shown the relationship to fatigue and HHV reactivation.

Research Activities

Development of biomarkers based on DNA methylation in the NCAPH2/LMF2 gene promoter region for diagnosing AD and amnesic mild cognitive impairment

For the early treatment of dementia, a convenient method of diagnosis with biomarkers is required for AD and amnesic mild cognitive impairment (aMCI). To examine differences in DNA methylation due to AD and aMCI, we performed genome-wide screening by measuring blood DNA methylation levels with the Infinium HD Methylation Assay (Illumina, Inc., San Diego, CA, USA) in 3 groups of 4 subjects matched for age and sex: healthy control subjects and patients with aMCI or AD. The genome-wide analysis produced 11 DNA methylation loci that distinguished the 3 groups. For confirmation, we increased group sizes and examined samples by pyrosequencing, which revealed that DNA methylation in the non-SMC condensin II complex subunit H2 gene (*NCAPH2*)/lipase maturation factor 2 gene (*LMF2*) promoter region was significantly lower in patients with AD (n = 30) or aMCI (n = 28) than in healthy control subjects (n = 30) (P < 0.0001, analysis of covariance). No association was found between methylation levels and the apolipoprotein E genotype. We believe that the *NCAPH2/LMF2* methylation level might be a convenient and useful biomarker for diagnosing AD and aMCI.

Genetic association between presenilin 2 polymorphisms and AD and dementia of Lewy body type in a Japanese population

Background/Aims: Mutations of the presenilin 2 gene (*PSEN2*) cause familial AD. Common polymorphisms affect gene activity and increase the risk of AD. Nonsynonymous polymorphisms in the *PSEN2* have clinically shown Lewy body dementia (LBD) phenotypes. Therefore, we investigated whether *PSEN2* polymorphisms are associated with AD or LBD.

Methods: Seven single nucleotide polymorphisms (SNPs) of *PSEN2* were analyzed with a case-control study of 288 patients with AD, 76 patients with LBD, and 105 age-matched control subjects.

Results: Strong linkage disequilibrium was found from rs1295645 to rs8383 of *PSEN2*. The SNPs and AD onset were not associated, and genetic associations between AD and *PSEN2* were not detected. Although the number of cases was small, the SNPs studied did not modify the risk of LBD developing.

Conclusion: The common SNPs of *PSEN2* did not affect the risk of AD or LBD in a Japanese population. Because genetic variability of *PSEN2* is associated with behavioral and psychological symptoms of dementia in AD and LBD, further detailed analyses of the behavioral and psychological symptoms of dementia of both diseases should be performed.

Molecular mechanism of depressive disorder caused by HHV-6 latent infection

Background: Although stress is a major risk factor for depressive disorder, how stress induces depression is poorly understood. In our previous study, we showed that salivary HHV-6, which may invade the brain via the olfactory pathway, is increased by stress. Furthermore, we have identified small protein encoded by the intermediate stage transcript of HHV-6 (SITH) 1, which is produced specifically in the astrocytes during HHV-6 latency, and have found that patients with depression have antibodies to SITH-1.

Objective: To examine whether HHV-6 SITH-1 production in the olfactory system, which may be enhanced by stress, causes depressive disorder and to reveal the molecular mechanism by which SITH-1 induces depression.

Methods: A recombinant adenovirus carrying glial fibrillary acidic protein promoterdriven SITH-1 (SITH-1/Adv) was inoculated intranasally into C57BL/6 mice. A recombinant adenovirus without SITH-1 (control/Adv) was used in the control experiment. One week later, the tail suspension test was performed to assess the depressive-like behavior. Twenty-four hours later the olfactory bulb and brain were harvested for gene expression analysis. Depression-related messenger (m) RNAs were quantitated with the real-time reverse transcriptase-polymerase chain reaction.

Results and Discussion: In SITH-1/Adv mice, SITH-1 was detected with immunofluorescent staining in the olfactory epithelium. In the tail suspension test, immobility time was significantly greater in SITH-1/Adv mice than in control/Adv mice. The increase in immobility time was suppressed by pretreatment with an antidepressant agent (fluoxetine).Inoculation with SITH-1/Adv significantly increased expression of corticotropinreleasing hormone mRNA and, interestingly, significantly decreased bcl-2 mRNA and increased apoptotic cells (as indicated by terminal deoxyribonucleotidyl transferasemediated deoxyuridine triphosphate-fluorescein nick-end labeling) in the olfactory bulb. Overall, stress induces HHV-6 SITH-1 production in the olfactory system and subsequently induces brain cell apoptosis and corticotropin-releasing hormone overexpression, which may ultimately cause depressive disorder.

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Department of Bacteriology

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General Summary

Research projects of our department have focused on: (1) a novel single point mutation in domain 2 of the stress-inducible sigma factor (S (RpoS) attenuates RpoS activity, (2) a straightforward assay for measuring glycogen levels and RpoS activity, (3) the role of gut microbes on the host nitrogen cycle, (4) the molecular mechanisms of type 8 secretion system, (5) an exploration of novel physiological functions of polyphenols, and (6) the analysis of staphylococcal biofilm dispersal.

Research Activities

A novel single-point mutation in domain 2 of the stress-inducible RpoS attenuates its activity

RpoS regulates stress resistance genes in *Escherichia coli*, such as the *katE* encoding catalase hydroperoxidase (HPII) and the *glg* encoding glycogen synthesis protein. 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 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 proline, which can destroy the alpha-helix structure at 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 to combine the ease and simplicity of qualitative approaches. The assay reagent comprised 2% iodine solution (2% iodine/1 M NaOH), and the basic principle of this assay is the iodine-glycogen reaction, which produces a reddish brown color that can be measured using a spectrophotometer. A calibration plot using the known amount of glycogen yielded the best linear fit over a range of 10-300 µg/assay ($R^2 = 0.994$). 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.

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 used by organisms. Compared with 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 investigated the roles of gut microbes on the nitrogen cycle in hosts.

Molecular mechanisms of type 8 secretion system

Previously, genome-wide screening of genes involved in the type 8 secretion system (T8SS), which secretes and assembles extracellular amyloid fibrils, termed curli, on the surface of bacteria identified the highly conserved molecular chaperone 70 kilodalton heat shock protein (HSP 70 or DnaK). The protein DnaK is known to play important roles in quality control of diverse cytoplasmic proteins, membrane proteins, and secretion proteins. The aim of this study was to clarify molecular mechanisms by which DnaK regulates the expression and quality of T8SS. Mutational analysis combined with in-cell protein folding and cell-free protein folding analyses demonstrated that DnaK engages in the folding of some transcriptional regulators involved in the expression of T8SS-related genes. Comprehensive approaches will provide mechanistic insights into the quality control of T8SS by DnaK and may lead to an understanding of the regulation of severe neurodegenerative diseases caused by protein misfolding, such as Alzheimer's disease and prion disease.

Exploration of novel physiological functions of polyphenols

Exploration of the potential functions of food constituents provides an additional value for health and offers applications for preventing diseases. In this study, we sought to identify small natural compounds that inhibit bacterial biofilm formation without attenuating growth. We found that myricetin, a type of polyphenol, effectively prevented biofilm formation by *E. coli* and *Staphylococcus aureus*, including methicillin-resistant strains, in a dose-dependent manner. Myricetin inhibited production of curli, presumably via inactivation of DnaK. In addition, a more effective myricetin-derivative with approximately 10-fold higher activity than myricetin was identified. Its mode of action is now being elucidated.

Analysis of staphylococcal biofilm dispersal

In the staphylococcal development of biofilm, the bacteria formed the biofilm within 8 hours; however, the biofilm was dispersed after 24 hours. Analysis of the extracellular matrix of the biofilm showed that this dispersal correlated with nucleic acids being degraded in the matrix. The culture supernatant from dispersed biofilm caused the biofilm to disassemble. The fraction of 50 to 10 KDa was applied to the cationic exchange col-

umn, and the active fraction showed nuclease activity in DNA zymography. The dispersion of the biofilm was not detected in a *nuc* mutant, which suggested that nuclease is a key factor for the biofilm disassembly mechanism.

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Department of Tropical Medicine

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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

Possible cause of red meat allergy by tick bites

Recent several studies suggest that patients in the eastern United States with red meat allergy have serum immunoglobulin (Ig) E against ticks. The IgE recognizes galactose- α -1,3-galactose (α -Gal), which exists in ticks and in beef and pork, i.e., red meat. Japanese spotted fever, caused by Rickettsia japonica and transmitted via the tick Haemaphysalis longicornis, is endemic in Shimane Prefecture. Interestingly, the same area has many patients with red meat allergy. Therefore we collaborated with the Department of Dermatology, Shimane University School of Medicine, to examine the association of tick bites with red meat allergy. The carbohydrate α -Gal was detected in the salivary gland protein of *H. longicornis*, maintained for more 30 years in a laboratory, by immunoblotting with an anti- α -Gal antibody. The reactive several bands were approximately 50 kDa, whereas those of beef were 250 kDa or greater. Approximately 50 kDa of salivary gland proteinspecific IgE was detected in the sera of 24 of 30 patients with the allergy. These IgE bound several bands of different molecular weight in beef protein. The binding was lost after the blotted membrane was treated with sodium periodate and suggests that the recognition of IgE includes carbohydrates. These results suggest that sensitization to tick salivary gland protein containing α -Gal is also a cause of red meat allergy in Japan. This result implies that tick bites transmit several pathogens and also cause red meat allergy.

Development of novel maggot debridement therapy with genetically modified blowflies

Maggot debridement therapy is a biotherapy technique in which sterile *Lucilia sericata* maggots are applied to ulcers. Although the precise mechanisms by which maggots heal ulcers are still unknown, numerous successful cases, and some less-successful cases, have been reported. Therefore, performing maggot debridement therapy with genetically modified maggots — specifically, maggots that have enhanced feeding and expanded targets — was recognized as a way to establish a novel technique for treating a wider range of cases more quickly. First, the relation between the olfactory mechanisms and feeding activity of

maggots was analyzed. The olfactory perceptions are speculated to differ between fieldcaught wild *L. sericata* and laboratory strains because of their feeding targets, various vertebrate remains, including the human body and ground beef/pork. Three independent lines were established by crossing field-caught blowflies, both morphologically and genetically identified, in each collected group. One of the olfactory-related genes, odorant receptor coreceptor (*Orco*), which is expressed in all olfactory receptor cells, was determined and analyzed for its sequences in each established fly strain and laboratory strain. All amino acid sequences of *Orco* were conserved and suggested a lack of diversity attributed to flies' feeding targets. As the next step to establish a novel method of maggot debridement therapy, genetically modified *L. sericata* maggots, based on the obtained sequence of *Orco*, are being produced.

Elucidation of molecular basis of tick host detection

Tick-borne diseases represent 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. Mosquitoes are known to have a remarkable ability to locate blood meals using host body emanations, such as CO₂, smell, and heat acting as strong mosquito attractants. Recently, evidence for thermosensitive sensilla on mosquito appendages has been uncovered. The activation of a transient receptor potential, an ion channel involved in various types of sensory reception, including thermoreception, chemoreception, mechanoreception, and photoreception, was reported to be caused by an increase in temperature in mosquitoes from 25°C to 37°C. In contrast, tick forelegs are antennae necessary for recognizing distant hosts with 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 effects of ambient temperature on locomotion in the larval, nymph, and adult stages.

Dissection of the blood-sucking behavior of mosquitoes

Exploring the molecular mechanism of the blood-sucking behavior of female mosquitoes is a critical step in fighting against vector-borne diseases, such as dengue and malaria, because pathogens are transmitted when mosquitoes are gorging on blood. In blood, ATP is known to serve as a phagostimulant. To confirm the nature of phagostimulants, we examined whether other nucleotides can be sucked by mosquitoes. Both AMP and deoxy-AMP served as phagostimulants like ATP, while adenosine and guanosine triphosphate did not. This finding suggests the existence of a chemoreceptor that specifically recognizes adenine nucleotides. To isolate candidate chemoreceptors of ATP, we performed RNA sequencing analyses of mosquito midguts to compare the gene expression pattern of pre- and post-ATP/blood suction. Changes in the expression of several molecules were common between ATP and blood suction, suggesting that these molecules are chemoreceptors of adenine nucleotides in the midgut of mosquitoes.

Revisiting a method for diagnosing toxoplasmosis: Development of the Toxoplasma *Kill-ing Observation test*

Toxoplasma gondii, the most successful protozoan, infects approximately one-third of

persons worldwide. In most people who are infected, except immunocompromised patients and pregnant women, toxoplasmosis is a self-limited disease with mild symptoms or no symptoms. Immunocompromised patients, such as those who have acquired immunodeficiency syndrome, have undergone organ transplantation, or use steroids, are at risk for Toxoplasma encephalitis, pneumonitis, and retinitis. Many types of serodiagnosis are widely used to detect toxoplasmosis around the world. However, because Toxo*plasma* immunoglobulins G and M are the only available serodiagnostic agents in Japan, diagnosis is complicated in some cases. The Sabin-Feldman dye test, which was reported in 1948, is used to evaluate the aggregate ability of the tachyzoite-cidal immunoglobulin titer in a patient' serum. This classic serodiagnostic test still has high sensitivity and specificity as a standard diagnosis. A problem with the dye test is the complicated evaluation method, in which stained tachyzoites must be counted through visual recognition. We examined this problem with a tachyzoite expressing green fluorescent protein, which is an alternative marker for evaluating the deactivation of tachyzoites. The new, improved dye test, the Toxoplasma Killing Observation test, has the advantages of its objectivity and retention for evaluation and achieves outcomes equivalent to those of the classic Sabin-Feldman dye test.

Publications

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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 2015 academic year focused on: (1) effects of nanomaterials on chromosomal abnormality; (2) effects of Zn-deficiency on the expression of interleukins (ILs) associated with a decrease in anti-inflammatory M2 macrophages; (3) mechanisms responsible for tubulointerstitial nephropathy induced by fluoride; (4) mechanisms of a developmental stage-specific toxicity of dioxins; (5) molecular approaches toward cancer chemoprevention with food factors; (6) effects of arsenic on the cholesterol metabolism; (7) the decompression stress in the hyperbaric work; (8) menopause-specific health literacy; (9) help-seeking intentions for mental illness; (10) association between differences in blood pressure or HbA1c variability and the risk of cardiovascular disease in patients with type 2 diabetes; and (11) 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 found that ZnO_2 and AlO_2 nanoparticles significantly induced micronuclei 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 of Zn-deficient rats: Roles of IL-4 and IL-13

A deficiency of Zn causes growth retardation and dysfunction of the immune and reproductive systems. We found that messenger RNA expression of IL-4 and IL-13, which induce differentiation into M2 macrophages, in splenic lymphocytes was significantly lower in rats with a Zn-deficient diet than in rats with a standard diet. Lymphocytes positive for IL-4 and IL-13 in the spleen were fewer in rats with a Zn-deficient diet than in rats with a standard diet.

3. Potential mechanisms responsible for tubulointerstitial nephropathy induced by fluoride in rats with unilateral ureteral obstruction

Fluoride, an environmental pollutant, is excreted from the kidney. The toxic effects of fluoride may deteriorate in animals with impaired renal function. In our previous animal experiments, ICR-derived glomerulonephritis mice, which have impaired renal function, were more severely affected by fluoride. In this study, we used the tubulointerstitial fibrosis model (unilateral ureteral obstruction) to examine whether fluoride deteriorates tubulointerstitial nephropathy in rats with unilateral ureteral obstruction.

4. Elucidation of the mechanism of a developmental stage-specific toxicity of dioxins Dioxin-induced hydronephrosis develops only a few days after birth in rodents. The molecular basis of this phenomenon has long been unknown. Aryl hydrocarbon receptor transactivation and prostaglandin E_2 overproduction are causes of dioxin-induced hydronephrosis, and only prostaglandin E_2 overproduction was found to be absent in dioxin-exposed adult mice. This finding indicates that the aberrant up-regulation of prostaglandin E_2 synthesis is responsible for this developmental stage-specific toxicity of dioxins. 5. Molecular approaches toward cancer chemoprevention with food factors

Carcinogenesis is closely related to lifestyle, including eating habits. We have attempted to establish an evidence-based cancer prevention method using food factors, including phytochemicals and trace elements. We found 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 with up-regulation of p21 and induced apoptosis via an intrinsic pathway.

6. The effect of arsenic on cholesterol metabolism

Recent epidemiological studies suggest that arsenic exposure is involved in atherosclerosis. In this study, we focused on the effect of arsenic on cholesterol metabolism by using Hepalclc7 cells. Gene expression analysis showed that arsenic suppresses the expression of ATP-binding cassette transporter A1, which is involved in high-density lipoprotein efflux. To elucidate the mechanisms of inhibition of this transporter, we are now focusing on the liver X receptor pathway.

7. A study of decompression stress in hyperbaric work

The decompression stress from hyperbaric work has been evaluated with the Doppler bubble detection technique. We attempted to evaluate the decompression stress by means of human herpes virus (HHV) 6 in saliva and the Doppler technique. The number of HHV-6 DNA in saliva was well correlated with the results of Doppler bubble detection. We were able to use HHV-6 in saliva as a marker to evaluate decompression stress.

Epidemiology, evidence-based medicine, investigation, and medical informatics 1. Menopause-specific health literacy

An Internet-based survey was conducted among Japanese women aged 30 to 59 years. The majority (85%) of participants correctly labeled the vignette as menopausal symptoms, and 60% expressed an intention to seek medical care if they had the presented symptoms.

2. Help-seeking intentions for mental illness

An Internet-based survey was conducted among Japanese adults aged 20 to 59 years. Those living in a communicative neighborhood were significantly more likely to express informal and formal help-seeking intentions.

3. Relationships between the risk of cardiovascular disease in patients with type 2 diabetes and both visit-to-visit variability and time-to-effect differences in blood pressure

Visit-to-visit variability in blood pressure is predictive of the incidence of cardiovascular disease independently of mean blood pressure in patients with type 2 diabetes. Increased systolic blood pressure over the preceding 3 to 5 years indicated a significant risk for cardiovascular disease.

4. The combined effect of visit-to-visit variability in HbA1c and systolic blood pressure on the incidence of cardiovascular events in patients with type 2 diabetes

Long-term visit-to-visit variability in HbA1c and systolic blood pressure simultaneously represented a combined and additive risk for the incidence of cardiovascular disease in patients with type 2 diabetes. In addition, a synergistic effect was suggested to exist between HbA1c variability and mean systolic blood pressure levels for the incidence of cardiovascular disease.

5. Effects of polaprezinc on pica and polydipsia

We performed an open-label trial to evaluate the effects of polaprezinc in patients who had pica with or without polydipsia and to examine the changes in serum concentrations of brain-derived neurotrophic factor before and after polaprezinc treatment. The trial is in progress.

6. 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 a binge-eating disorder. We also assessed the Eating Disorder Examination Questionnaire, the 16 Item Quick Inventory of Depressive Symptomatology Self-Report Version, body weight, blood biochemistry, and plasma concentrations of brain-derived neurotrophic factor over the course of the trial. The trial is in progress.

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Department of Forensic Medicine

Kimiharu Iwadate, Professor Kenji Fukui, Assistant Professor Kentaro Sakai, Associate Professor Kyoko Maebashi, Assistant Professor

General Summary

Our research projects in 2015 have 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. The relation between dangerous drugs and coronary constriction

A recent problem has been the abuse of dangerous drugs, including cannabis and amphetamines, in which a part of the chemical structure has been modified. However, what is not fully known about these drugs is their effect on humans, their lethal doses, and their longterm behavior in the body. We analyzed the findings of forensic autopsies of 4 persons who were suspected of having used dangerous drugs just before death. Of these persons, 3 had coronary atheromatous lesions and 2 were in their 20's. Therefore, our findings suggest that the abuse of a dangerous drug can produce coronary lesions.

DNA analysis

1. Identification of war-dead remains with DNA analysis

We performed identification of war-dead remains buried in the former Soviet Union by means of DNA analysis as part of the war-dead remains return project of the Ministry of Health, Labour and Welfare. For genetic markers we used single nucleotide polymorphisms of hypervariable regions of mitochondrial DNA and short tandem repeats of nuclear DNA.

2. DNA extraction from chewing gum

We have investigated a method of extracting high-purity DNA from chewing gum. In addition, we showed that there was no correlation between chewing time and the DNA yields.

3. The detection and analysis of X chromosome short tandem repeats locus

The analysis of short tandem repeats (STRs) located on the X chromosome is useful for kinship testing. We performed detection and population genetic study of a novel tetranucleotide X chromosomal STR (X-STR) locus in the present study. We analyzed the sequence structures of novel X-STRs, the appearance frequency of alleles, and forensic statistics data. We registered this data with the International Nucleotide Sequence Database Collaboration. We are planning to investigate the relevance with other X-STRs by linkage analysis.

Forensic toxicology

1. Quantitative analyses of medicines and poisonous substances

Medicines and poisonous substances (abused drugs, alcohol, carbon monoxide, cyanide, and agricultural chemicals) suspected to have caused deaths were quantitatively analyzed with gas chromatography, gas chromatography/mass spectrometry, and spectrum photometry in tissue specimens obtained at autopsy.

2. Examination of a method for analyzing dextromethorphan and tadalafil

We detected dextromethorphan, which is a cough medicine, and tadalafil, which is a medicine for treating erectile dysfunction, in autopsy cases. Qualitative and quantitative methods of analyzing these medicines were examined with gas chromatography/mass spectrometry.

3. Construction of drug screening method

A drug screening method was constructed with liquid chromatography-tandem mass spectrometry. About 270 kinds of drugs can be targeted, but we plan to increase the number of targets. The addition of target drugs has been considered.

4. Analysis of hydrogen sulfide and metabolites

Hydrogen sulfide or its metabolites have reportedly been detected at autopsy when the cause of death is not hydrogen sulfide poisoning. From bodies in which sulfide poisoning was not the cause of death and from healthy individuals, samples of blood and urine were collected and analyzed for sulfide ions and thiosulfate ions. The presence or absence and the aging of sulfide ions and thiosulfate ions were examined.

In the blood and urine from healthy individuals, generally sulfide ions and thiosulfate ion are hardly detected. However, slightly sulfide ion and thiosulfate ion were detected in this analysis. Sulfide ion was many in samples of blood stored for a long time. Sulfide ion is believed to be present owing to the protein degrading via corruption. In contrast, the concentration of sulfide ion in long-term storage samples of urine tended to be low. For thiosulfate ion, trends in both the blood and urine were unclear.

Radiocarbon analysis

1. Establishment of date of birth

We studied estimating the date of birth from carbon-14 isolated from a tooth. To apply this method to forensic medicine, we have examined the minimum amount of enamel required for the analysis. We have found that the lower limit of enamel to be needed is approximately 60 mg.

Department of Internal Medicine Division of Gastroenterology and Hepatology

Senya Matsufuji, Professor Hirokazu Nishino, Professor Shigeo Koido, Associate Professor Tomohisa Ishikawa, Associate Professor Kazuhiko Koike, Assistant Professor Seiji Arihiro, Assistant Professor Hiroshi Abe, Assistant Professor Masayuki Saruta, Assistant Professor Akiyoshi Kinoshita, Assistant Professor Yoshio Aizawa, Professor Toshifumi Okusa, Professor Atsushi Hokari, Associate Professor Mika Matsuoka, Assistant Professor Satoshi Suto, Assistant Professor Yasushi Onoda, Assistant Professor Shinichiro Uetake, Assistant Professor Kan Uchiyama, 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. Areas under the receiver operating characteristic curves of the simple clinical colitis activity index, Mayo endoscopic scoring, and Matts' grading (histologic activity) for PEG-MUM were each higher than for C-reactive protein.

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

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

Levels of procalcitonin are relevant to immunologic responses that contribute to systemic inflammation responses and septic shock. Procalcitonin demonstrates the activity of chronic inflammatory and autoimmune diseases, such as Wegener's granulomatosis. Herein, we hypothesized that the serum procalcitonin level is helpful for predicting the disease activity of inflammatory bowel diseases: Crohn's disease, ulcerative colitis, or intestinal Behcet's disease. The serum procalcitonin levels were correlated to the activity of Crohn's disease and intestinal Behcet's disease but not ulcerative colitis. These levels were helpful to distinguish cases of Crohn's disease that are severely active to fulminant from cases that are mildly to moderately active and may serve as a new serological marker of disease activity, as does C-reactive protein.

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

We have developed a method for boosting cancer theranostics utilizing near-infrared light and monoclonal antibody-photoabsorber conjugates, both specific for human epidermal growth factor receptor 2.

3. A genetic factor associated with drug-induced leukopenia in Japanese patients with inflammatory bowel disease

The 94C>A mutation of the inosine triphosphate gene (*ITPA*) and genetic factors have been suggested to be involved in the onset of thiopurine-induced leukopenia in Japanese patients with inflammatory bowel disease.

4. The investigation of efficacy and adherence to an omega-3 diet for remission maintenance in patients with inflammatory bowel disease

The intake of omega-3 polyunsaturated fatty acids and the subsequent associated efficacy for the maintenance of remission may be achieved by understanding the importance of omega-3 diet therapy.

5. Clinical features of patients with early colonic cancer treated with endoscopic submucosal dissection

We evaluated the clinical features of patients with early colonic cancer treated with endoscopic submucosal dissection.

Liver

1. The development of targeting therapy for cancer stem cells in liver cancers

The only curative treatment for patients with primary liver cancers is surgical resection at an early stage. However, cancers are diagnosed in most patients at an advanced stage, 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 spalt like transcription factor 4 (SALL4) regulates cell fate decision in hepatic stem/ progenitor cells during normal liver development, is indicative of aggressiveness and a 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 therapeutic strategy against liver cancers.

2. The relationship between lipoprotein profile and cholesteryl ester transfer protein in patients with chronic hepatitis C

The concentration of cholesteryl ester transfer protein was significantly high in patients with chronic hepatitis C, and hepatitis C virus (HCV) infection was extracted as an independent factor. An examination of lipoprotein profiles of patients with HCV infection found that low concentrations of low-density lipoprotein-cholesterol and apolipoprotein C-II are associated with liver fibrosis and that a high concentration of apolipoprotein E is associated with the HCV genotype.

3. Clinical characteristics of autoimmune hepatitis

We evaluated clinical characteristics among 78 patients with autoimmune hepatitis onset before 2006 and 59 patients with onset since 2006. No significant differences were found between the groups.

4. Diseases of the hepatobiliary system in superelderly patients

The mean life expectancy in Japan has dramatically increased. Therefore, the opportunity to examine superelderly patients (80 years or older) who have diseases of the digestive

tract has significantly increased in our hospital. Thus, we have investigated the clinical characteristics and outcomes of superelderly patients with diseases of the digestive tract, including hepatocellular carcinoma, pancreatic cancer, chronic HCV infection, and acute cholecystitis.

5. Investigation of the Frailty Index in elderly patients with diseases of the digestive tract We evaluated whether simplified Frailty Index scores are associated with clinical outcomes or adverse outcomes after treatment in elderly patients (older than 80 years) with diseases of the digestive tract.

6. The efficacy of tolvaptan in patients with cirrhosis and refractory ascites

Tolvaptan, a novel oral arginine vasopressin V2 receptor antagonist, has been available since September 2013 for treating patients with hepatic edema. We evaluated the therapeutic efficacy and safety of tolvaptan administration in patients with cirrhosis and refractory ascites.

Pancreas

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

Prolonged low plasma levels of interleukin 6 and interleukin 8 in patients with pancreatic ductal adenocarcinoma may be a prognostic marker for the clinical outcome of chemoimmunotherapy.

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Department of Internal Medicine Division of Neurology

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General Summary

Our clinical research in 2015 was conducted in the following areas: (1) cerebrovascular disease, (2) neurodegenerative disease, and (3) autoimmune disease.

Research Activities

Clinical researches

Cerebrovascular disease

1. Patients with hyperacute stroke associated with aortic dissection

To avoid administering a tissue plasminogen activator to patients with stroke and aortic dissection, the aortic dissection score should be used to make prehospital management more adequate.

2. Comparative study of transcranial Doppler examination and a novel probe for diagnosing patent foramen ovale

An examination with a pasteable soft ultrasound probe at the common carotid artery can be used to evaluate the presence of a large patent foramen ovale more precisely than can a transcranial Doppler examination.

3. Analysis of cerebral microbleeds after acute-phase treatment of a lacunar infarction Acute-phase treatment rarely increases the number of cerebral microbleeds in patients with lacunar infarction and branch atheromatous disease.

4. Complications of novel oral anticoagulants in patients with acute cerebral infarction Careful neurological examinations are needed after novel oral anticoagulants have been administered to patients with acute cerebral infarction.

5. Thromboembolic complications of coil embolization for unruptured brain aneurysm Factors that tended to be related to symptomatic infarction were a low volume embolization ratio, a wide-necked aneurysm, and a long operative time.

6. Transcranial color-coded sonography of the vertebral artery for the diagnosis of right-to-left shunts

Neurodegenerative disease

1. Cardiovascular autonomic dysfunction in patients with Parkinson's disease and related disorders

Patients with reduced nocturnal blood pressure fall were shown with ¹²³I-metaiodobenzyl-

guanidine scintigraphy to have a low cardiac uptake. Nocturnal blood pressure fall may improve in some patients who have received a dopamine agonist.

2. Evaluation with a portable gait rhythmogram of gait disturbance in Parkinson's disease The portable gait rhythmogram has high sensitivity for estimating clinically important deficits of hypokinesia and gait bradykinesia.

3. White matter lesions are correlated with olfactory dysfunction in early Parkinson's disease

Olfactory dysfunction was highly correlated with white matter lesions. This finding suggests that the pathogenesis of olfactory dysfunction is associated with that of white matter lesions in Parkinson's disease.

4. Quantitative motion analysis with a portable gait rhythmogram after cerebrospinal fluid is drained from patients with idiopathic normal pressure hydrocephalus

A portable gait rhythmogram can easily be used to analyze long-duration gaits in daily life and to provide additional quantitative data on former measurements of cerebrospinal fluid drainage.

5. Survey of prodromal symptoms of Parkinson's disease in Japan

6. Thyroid hormone level is associated with motor symptoms in early Parkinson's disease The thyroid hormone level, especially the free triiodothyronine level, is closely related to motor symptoms in patients with early Parkinson's disease.

7. Clinical characteristics of supine hypertension in early Parkinson's disease

Regarding global cognitive decline, supine hypertension is a far riskier comorbidity of early-stage Parkinson's disease than is orthostatic hypotension.

8. Body weight and dysautonomia in early Parkinson's disease

Dysautonomia may play a partial role on weight variation in the early stage of Parkinson's disease.

Depression is associated with abnormal nocturnal hypotension in early Parkinson's disease

10. Hyponatremia and fasting hypoglycemia in Parkinson's disease

11. Dysphagia and vocal cord palsy in multiple system atrophy

In patients with multiple system atrophy and vocal cord palsy, percutaneous endoscopic gastrostomy was enforced safely with noninvasive positive pressure ventilation therapy.

12. Dysphagia in Parkinson's disease and dementia with Lewy bodies

Autoimmune disease

1. Findings of single photon emission computed tomography after human papillomavirus vaccination in Japan

Various clinical symptoms in patients with human papillomavirus vaccine associated with neuroimmunopathic syndrome were caused by central nervous system impairment after vaccination.

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Department of Internal Medicine Division of Nephrology and Hypertension

Takashi Yokoo, Professor Makoto Ogura, Associate Professor Kazushige Hanaoka, Assistant Professor Nobuo Tsuboi, Assistant Professor Yukio Maruyama, Assistant Professor Keitaro Yokoyama, Associate Professor Yoichi Miyazaki, Associate Professor Masato Ikeda, 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 of immunoglobulin A nephropathy

We demonstrated that tonsillectomy and histological grade indicate a lower risk of recurrence in patients with immunoglobulin A (IgA) nephropathy. Studies have been published of patients with IgA nephropathy showing acute exacerbations after long-term courses and the analysis of elderly patients with IgA nephropathy.

Studies of low glomerular density in CKD

Our studies showed that low glomerular density was strongly associated with the prognosis of various diseases. Collaborative research about the estimation of nephron numbers in Japanese is in progress.

Studies on podocyte damage

Transgenic mice (NEP25) express human CD25 (hCD25) selectively on podocytes, and injection of hCD25-targeted recombinant immunotoxin permits selective injury only to podocytes. We generated mosaic mice that contain both hCD25⁺ and hCD25⁻ podocytes. The immunotoxin sequentially induced hCD25⁻ podocyte injury, and this effect was dominant, especially in male mice.

Basic study of kidney regeneration

We demonstrated the construction of urine excretion pathways in rats. Rat metanephroi or metanephroi with bladders (developed from cloacas) were transplanted into host rats. His-topathologic analysis showed that tubular lumina dilation and interstitial fibrosis were less common in kidneys developed from cloacal transplantation than in kidneys developed with metanephroi transplantation. We then connected the host animal's ureter to the cloa-

cal-developed bladder, a technique we called the stepwise peristaltic ureter (SWPU) system. The application of the SWPU system avoided hydronephrosis and permitted the cloacas to differentiate well, with cloacal urine being excreted persistently through the recipient ureter. Finally, we demonstrated a viable preclinical application of the SWPU system in cloned pigs. In the pig study the SWPU system also inhibited hydronephrosis.

Studies of CKD mineral and bone disorder

We investigated the epigenetic modifications in the parathyroid glands of CKD-mineral and bone disorder, then we have reported modifying DNA methylation patterns in parathyroid glands with CKD-mineral and bone disorder. We then analyzed the glial cells missing homolog 2 gene (Gcm2) in parathyroid glands, which is the essential transcription factor for parathyroid development in terrestrial vertebrates. Furthermore, we conduct a biological functional analysis of *Gcm1*, an ortholog of *Gcm2*, in the kidney. The magnesium concentration is a proven predictor of mortality in hemodialysis patients. We showed in a prospective cohort study that the use of a proton pump inhibitor is associated with an increased risk of hypomagnesemia in patients with hemodialysis. To clarify the association between all-cause mortality and the magnesium concentration, including fibroblast growth factor 23, we are now conducting a cohort study. Using annual surveys of dialysis facilities throughout Japan managed by the Japanese Society for Dialysis Therapy we have also identified the associations between higher serum ferritin levels and higher rates of all-cause and cardiovascular mortality among 191,902 patients who are undergoing hemodialysis and peritoneal dialysis.

Studies of peritoneal dialysis

With outcomes of combined therapy with peritoneal dialysis and hemodialysis in a cohort of more than 100 patients we confirmed the availability of combined therapy. Moreover, we found that the survival outcome with combined therapy was not worse than that with peritoneal dialysis or hemodialysis alone. We are using laparoscopy to evaluate peritoneal injury.

Study of renal transplantation

We participated in the Japan Academic Consortium of Kidney Transplantation and investigated Alport syndrome and IgA vasculitis in patients with kidney transplantation. We found that Alport syndrome did not recur and that the rates of allograft and patient survival were similar to those of other diseases. In cases of IgA vasculitis, we found that the recurrence rate was 28.6% and that recurrence itself did not affect allograft survival. This result could be an effect of tonsillectomy plus corticosteroid pulse therapy. In addition, we found that the single nucleotide polymorphism of the glucocorticoid induced 1 gene (*GLCCI1*) could be related to posttransplant hypertension.

Renal protective effects of T-type calcium channel blockade via the blood-brain barrier in CKD model rats

We investigated whether the mechanism of a renal protective effect differs because of an agent's ability to penetrate the blood-brain barrier. A new T-type calcium channel

blocker, which can or cannot penetrate the blood-brain barrier, was examined.

Renal protective effects of azilsartan in a rat model of adenine-induced renal failure Although daily urinary sodium excretion was decreased in nonmedicated rats, azilsartan lead to fewer decreases in sodium excretion, urinary protein excretion, and sympathetic nerve activity. We will investigate the molecular mechanism of the renal protective effects by the suppression of sympathetic nerve activity.

A lower serum level of uric acid is associated with rates of all-cause and cardiovascular mortality among patients receiving hemodialysis in Japan

We investigated the relationship of the serum level of uric acid to the rates all-cause and cardiovascular mortality in patients receiving hemodialysis. Lower serum levels of uric acid were independently associated with the rates of all-cause and cardiovascular mortality among patients receiving hemodialysis. A close monitoring of the serum level of uric acid is believed to be necessary to assess patients receiving hemodialysis.

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Department of Internal Medicine Division of Rheumatology

Daitaro Kurosaka, Professor Ken Yoshida, Assistant Professor Isamu Kingetsu, 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. Power Doppler ultrasonography for detecting abnormal fascial vascularity: a potential early diagnostic tool in fasciitis of 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.

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.

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.

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

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. 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, from catheterization examinations and treatments in patients with ischemic heart disease, into our large and precise database. Using this database, we have been performing a study comparing risk factors, clinical outcomes, and other data. We have recently been studying 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. 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.

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

A database has been established with respect to the clinical data of approximately 3,000 cases of patients admitted to hospital for the purpose of cardiac catheter examination and treatment, and the relationships among factors, which are difficult to analyze with standard multivariate analysis alone, are being analyzed with a highly statistical analysis. Currently, covariance structure analysis is used for ongoing research related to the plasma concentration of B-type natriuretic peptide (BNP). Regarding BNP, detailed analytic results have been acquired from various areas, such as the relationship between the change in BNP concentration and the change in body weight before and after the treatment of acute hear failure and also the relationship between BNP concentration and anemia in patients with heart failure. These results are promoting the publication of articles. Moreover, we are continuously trying to elucidate the mechanism of the clinical findings through basic research.

Imaging Research Group

As for coronary artery spasm, which are common in ischemic heart disease in Japanese patients, vascular tonus changes in coronary arteries were studied through image pathognomy by coronary computed tomography (CT). In addition, since CT is becoming more valuable as a test for evaluating the aortic valve condition before transcatheter aortic valve replacement (TAVR), a recently-stated approach to treatment of aortic stenosis, we have been aggregating the valuable information. In other imaging methods, such as echocardiography, heart magnetic resonance imaging (MRI), and myocardium isotope testing, clinical research topics are still being searched and analyzed with other subjects, such as cardiac myopathy and abnormal cardiac rhythm.

Molecular Biology Research Group

Glucose becomes an important preferential substrate for cardiac metabolism and ATP generation during ischemia-reperfusion injury (IRI). A series of our recent studies on insulin signaling indicated that a transient increase in glucose uptake into myocardium is protective against IRI. The study from the clinical database showed a transient decrease in the serum potassium level during acute coronary syndrome (ACS) attack, the degree of which is positively correlated with the plasma glucose level but not with homeostasis model assessment insulin resistance or HbA1c, as the indicators of insulin resistance. These data suggest the presence of endogenous glucose-coupled potassium lowering mechanisms, other than insulin, promoting glucose metabolism during ACS. The coupling of sodium-glucose co-transporter 1 (SGLT1) and Na⁺/K⁺ ATPase is a potential mechanism. In fact, a study of Langendorff 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 new insight into 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 (Δ K210 knock-in mouse) (B6;129-Tnnt2^{tm2Mmto}), we investigated how thrombin is involved in the development of DCM. We observed that thrombin expression was stronger in mice with DCM than in wild-type mice. We assessed the effects of a

direct thrombin inhibitor, dabigatran, in $\Delta K210$ 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.

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Department of Internal Medicine Division of Diabetes, Metabolism and Endocrinology

Kazunori Utsunomiya, Professor and chairperson Kuninobu Yokota, Professor Masami Nemoto, Associate Professor Rimei Nishimura, Associate Professor Hironari Sano, Assistant Professor Masaya Sakamoto, 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 expanded medical treatment with the Division of Diabetes, Metabolic and Endocrinology because of a rapid increase in the number of patients, mainly with diabetes and internal secretion diseases, such as those of the thyroid gland, pituitary gland, adrenal gland, the gonads.

By using such an increase in the number of patients as a basis, we are performing highquality studies.

Research Activities

Study on diabetes complications

- 1. Rho/Rho kinase signal and symptoms of very small blood vessels with diabetes
- 2. Rho/Rho kinase signal and symptoms of great vessels with diabetes

Study on epidemiology

- 1. Clinical study of diabetes with continuous glucose monitoring
- 2. Study of lifestyle-related diseases of local inhabitants and insulin resistance

Molecular biologic study of the pancreas's islets of Langerhans

Elucidation of the molecular mechanism change by obesity of the power of protein kinase C δ -dependent pancreas β -cell capacity change by the fat toxicity

Study on endocrinology

- 1. Fundamental researches
- 1) Study of mineral-corticoid receptor in diabetes
- 2) Study of Ca channel subclass and depression mechanism
- 3) Study of the potential transient receptor transient channel of fat cells
- 2. Clinical study
- Clinical study of adrenal tumors

Study of blood pressure variation

The main purpose of this study was to elucidate the mechanisms by which blood sugar levels, blood pressure, cholesterol, and changes in acylglycerol are related to the complications of diabetes and cardiovascular events.

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Department of Internal Medicine Division of Clinical Oncology/Hematology

Keisuke Aiba, Professor and chairperson 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 2015.

Research Activities

Leukemias

Many patients with previously untreated hematological disorders have been referred to our department. The disorders in 2015 included acute myeloid leukemia (AML), 26 cases; acute lymphoblastic leukemia (ALL), 9 cases; and chronic myeloid leukemia (CML), 6 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. The JALSG protocol studies performed in 2015 were as follows: JALSG-AML209-GS, JALSG-CBF AML209-KIT, JALSG-ALL-CS-12, JALSG-CS-11, JALSG-CML212, phaseIIJALSG-APL212, JALSG-APL212G, JALSG MDS212 (MDS212), JALSG-Ph(-) B-ALL213, JALSG Burkitt-ALL213, and JALSG Ph⁺ALL213. We also participated in several cooperative group studies and pilot studies: Aged Double-7 (newly diagnosed AML in the elderly: phase II), VEGA (MDS: phase II), a study of nilotinib (refractory CML: phase I/II), and a study of dasatinib (refractory CML: phase I/II).

Lymphomas

In 2015 we registered 81 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 a pivotal protocol study beginning in 2007. A randomized phase II study in patients with high-risk, diffuse, large B-cell lymphoma has also been started (biweekly rituximab, cyclophosphamide, hydroxy-daunorubicin, vincristine, and prednisone [bi-R-CHOP] \pm cyclophosphamide, cytarabine, dexamethasone, etoposide, and rituximab [CHASER] vs melphalan, cyclophosphamide,

etoposide, and dexamethasone [LEED]; JCOG0908). Other cooperative studies examined biweekly rituximab, etoposide, prednisone, vincristine, hydroxydaunorubicin (R-EPOCH: relapsed and refractory B-cell lymphoma: phase II) and pirarubicin, cyclophosphamide, vincristine, and prednisolone (THP-COP: newly diagnosed T-cell lymphoma: phase II).

Myeloma

We registered 11 patients with newly diagnosed multiple myeloma in 2015. A novel agent, the proteasome inhibitor bortezomib, became available in 2007, and we have used it with or without dexamethasone to treat patients who have refractory myeloma. A randomized phase II study was started in 2010 (JCOG0904) to evaluate the efficacy of bortezomib + dexamethasone versus thalidomide + dexamethasone in patients with relapsed or refractory chemoresistant multiple myeloma. A randomized phase II study evaluating melphalan + predonisolone + bortezomib (MPB) induction chemotherapy for elder patients and patients who refuse stem-cell transplantation was started in 2015 in patients with multiple myeloma (JCOG1105).

In-house protocols are also under investigation. These investigations include a phase II study of the cyclophosphamide + bortezomib + dexamethasone (CVD) regimen for patients with newly diagnosed multiple myeloma and a phase II study of the cyclophosphamide + lenalidomide + dexamethasone (CRD) regimen for patients with relapsed and refractory multiple myeloma.

Hematopoietic stem cell transplantation

To investigate and establish safer and more effective hematopoietic stem-cell transplantation, we have performed serial clinical studies examining umbilical cord blood transplantation with a bone marrow-nonablative procedure, a bone marrow-nonablative procedure using antithymic globulin, and mechanisms of graft-versus-host disease in hematopoietic stem-cell transplantation.

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 fluorourcil (5-FU) (DCF regimen) for patients with locally advanced esophageal cancer. The study has been completed, and the results have recently been published. Successively an improved protocol was launched 3 years ago and is now being investigated. In collaboration with another department we have performed and completed a novel drug-development study of an orally decaying formulation of S-1 in patients with advanced gastric cancer; the new formulation of S-1 has now become available for 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). Because 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 FOL-FILI have also been performed. Because oral drugs are more convenient and safer, 5-FU is replaced by S-1 or capecitabine in such intravenous combination chemotherapy regimens as FOLFOX or FOLFILI, leading to the development of improved regimens of S-1 + oxaliplatin (SOX), capecitabine + oxaliplatin (XELOX), irinotecan + S-1 (IRIS), and capecitabine + irinotecan (XELIRI). Salvage therapies using regorafenib or trifluridine/ tipiracil (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. We have been investigating an improved method enabling minimal residual disease to be detected in patients with multiple myeloma. Because the 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 reactivation using patient's salivary juice and blood samples, in collaboration with the Department of Virology. The preliminary results were reported at the annual meeting of the Multinational Association of Supportive Care in Cancer in Miami, Florida (USA). Gene expression analysis has been examined in blood samples to clarify efficacy and adverse effects in patients who have esophageal cancer and are undergoing chemoradiotherapy. Because esophageal cancer is a severe, life-threatening disease, such a study would be of great consequence. Supportive care in cancer is also important for patients with malignant disease and has been studied by us for many years. Measurement of the urinary level of liver-type fatty acid binding protein (L-FABP) can be used to predict renal damage caused by cisplatin. Therefore, we have been attempting to determine whether the L-FABP level should be measured for the early detection of renal damage in patients undergoing cisplatin combination chemotherapy, such as DCF and gemcitabine + dexamethasone + cisplatin (GDP). The studies are vigorously in progress.

Publications

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Department of Internal Medicine Division of Respiratory Diseases

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

General Summary

We developed clinical and basic research concerning chronic obstructive pulmonary disease (COPD), bronchial asthma, pulmonary infection, pulmonary fibrosis, and lung cancer. Basic research should resolve clinical problems and clinical research should construct novel treatments. We specifically investigated the role of apoptosis, senescence, and autophagy in the pathogenesis of various lung diseases. Basic research focusing on the molecular mechanisms of pulmonary fibrosis, and COPD are in progress. We also collaborate with National Cancer Research Center concerning the detection of mutations of the epidermal growth factor receptor (EGFR) gene (*EGFR*) and exosome research.

Research Activities

Cellular senescence and autophagy in COPD

The cause of COPD is the noxious effects of tobacco smoke. Cigarette smoke-induced accelerated cell senescence has been widely implicated in the pathogenesis of COPD. The accumulation of damaged proteins and organelles are typical manifestations of cellular senescence, indicating the involvement of autophagy in the regulation of cell senescence in COPD. We found the involvement of insufficient p62-mediated selective autophagic clearance of ubiquitinated proteins in accelerated cellular senescence in the pathogenesis of COPD (Fujii S, et al, Oncoimmunology 1: 630-641, 2012).

Mitochondria are dynamic organelles and continuously change their shape through fission and fusion. The proper regulation of mitochondrial dynamics is crucial for the maintenance of functional mitochondria. We found that cigarette smoke extract induced mitochondrial fragmentation and mitochondrial oxidative stress, which were responsible for acceleration of cellular senescence in human bronchial epithelial cells. Mitochondrial fragmentation induced by knockdown of fusion proteins also increased mitochondrial reactive oxygen species production and percentages of senescent cells. 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 exosomes. Exosomes are extracellular vesicles that play important roles in cell-to-cell communication. MicroRNA have potential roles in cellular homeostasis and the pathophysiology of various diseases. We found that microRNA-201 suppresses the autophagy related 7 gene (*ATG7*) and autophagy and that such suppression

leads to fibrogenesis in airway walls of COPD (Fujita Y et al. J Extracellular Vesicles 2015).

Cellular senescence and autophagy in idiopathic pulmonary fibrosis

Recent studies have shown that senescence is a risk factor for the development of idiopathic pulmonary fibrosis (IPF). We have produced evidence that lungs with IPF show enhanced senescence with a concomitant increase of sirtuin 6 (SIRT6) expression in epithelial cells, including aberrantly re-epithelialized bronchial cells. Transforming growth factor β induces senescence by increasing p21 expression and also induces SIRT6 expression, and artificial overexpression of SIRT6 efficiently inhibits transforming growth factor β -induced senescence via proteasomal degradation of p21 in human bronchial epithelial cells. These findings shed light on the accelerated epithelial senescence in the pathogenesis of IPF with a possible regulatory role for SIRT6 (Minagawa S, et al. Am J Physiol Lung Cell Mol Physiol. 300: L391-401, 2011).

Autophagy has been shown to prevent cellular senescence caused by tunicamycininduced endoplasmic reticulum stress in human bronchial epithelial cells. Conversely, autophagy inhibition was sufficient to induce myofibroblast differentiation in lung fibroblasts. Cellular senescence as measured by p21 expression and senescence-associated β -galactosidase staining was observed in metaplastic epithelial cells covering fibrosing lesions. These findings suggest that insufficient autophagy is a potent underlying pathologic finding 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).

Etiologies of acute exacerbation of COPD in adults by real-time polymerase chain reaction

Respiratory infection is a major cause of exacerbation in COPD. Multiplex polymerase chain reaction (PCR) has recently been used to detect effectively both respiratory bacteria and viruses. To evaluate causes of the acute exacerbation of COPD, we used a rapid reliable process based on real-time (RT)-PCR for respiratory samples. We analyzed respiratory tract samples by comprehensive RT-PCR. We prospectively studied 46 patients with COPD and examined nasopharyngeal swabs and sputum samples. We performed RT-PCR designed to detect 6 bacterial species and 11 viruses. Viruses were detected in 17 (34%) of 50 exacerbations. The COPD exacerbations caused by Gram-negative bacilli were significantly associated with prolonged hospitalization for COPD exacerbations. We concluded that RT-PCR is useful for detecting the causes of infection and determining the risk of extended hospitalization (Shimizu K et al. Int J COPD 2015).

Analyzing cell-free plasma DNA by picoliter-droplet digital PCR to detect EGFR mutations in patients with lung adenocarcinoma

Resistance develops in patients with adenocarcinoma of the lung who received EGFRtyrosine-kinase inhibitor (TKI) therapy. Noninvasive monitoring of the secondary Thr790Met mutation in *EGFR* is necessary for precise treatment of lung adenocarcinoma. We examined TKI-sensitive mutations (L858R and inflame exon 19 deletions) and TKI- resistant mutations (i.e., Thr790Met) in cell-free plasma DNA using picoliter-droplet digital PCR cell-free plasma DNA analysis. We found that picoliter-droplet digital PCR enables noninvasive assessment of *EGFR* mutations that confer resistance to TKIs (Seki Y et al. Oncologist 2015).

Publications

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Reviews and Books

Fujita Y, Kosaka N, Araya J, Kuwano K, Ochiya T. Extracellular vesicles in lung microenvironment and pathogenesis. *Trends Mol Med.* 2015; **21:** 533-42.
Department of Internal Medicine Division of General Medicine

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

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 has been a strong desire for a change of focus in medical education, from hospital-based specialty care to first-line health care, including community health care. Thus, we are now planning the postgraduate and life-long training program of physicians to acquire skills for the general practice required in the community.

Division of General Medicine, The Jikei University Katsushika Medical Center

We treated 2 cases of dengue disease and presented reports of both cases.

We assessed the association between the serum levels of thiamine and insulin. Outpatients and inpatients with diabetes were enrolled from Katsushika Hospital and examined for disease duration, therapy, plasma glucose levels, HbA1c, and the serum insulin and thiamine levels. We have continued to recruit patients with diabetes and intend to analyze the association between serum insulin levels and thiamine levels.

Division of General Medicine, The Jikei University Daisan Hospital

1. Study of polymyalgia rheumatic

We found that levels of C-reactive protein are high in all patients with polymyalgia rheumatic and that matrix metalloproteinase 3 is elevated in more than 80% of patients. We can cause mild cases of polymyalgia rheumatic to enter remission by administering 10 mg of prednisolone. We can cure approximately 50% cases, and the other cases need to be continuously treated. Methotrexate is used in refractory cases but has a limited effect. 2. Study of sepsis

Measurement of procalcitonin, white blood cells, and C-reactive protein is limitation in the early diagnosis of sepsis. We have begun to measure the new marker presepsin.

3. Study of "do not attempt resuscitation"

We studied whether the "do not attempt resuscitation" order and the Physician Orders for Life-Sustaining Treatment (POLST) form are known in university hospitals. Almost all physicians and nurses are aware of the "do not attempt resuscitation" order, but about 60% were confused in actual cases. Only 2% know about the PLOST form.

Division of General Medicine, The Jikei University Kashiwa Hospital

Our research at The Jikei University Kashiwa Hospital has consisted of 3 parts. The first was to develop interprofessional work in the Kashiwa area. We have conducted open seminars related to general medicine and clinical ethics 3 to 4 times per year.

The second part was to develop educational tasks for teaching medical students and junior physicians. We developed the education system using the Internet (called e-portfolio), and it is now being used.

The third part was to establish and manage a Hospital Ethics Committee and a system for Clinical Ethics Consultation at The Jikei University Kashiwa Hospital. There were 14 cases of consultation last year, and these cases are now being reconsidered for presentation and publication.

This year, we studied the use of the POLST form in Japanese hospitals.

Publications

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Department of Psychiatry

Kazuhiko Nakayama, Professor Kei Nakamura, Professor Hironari Sue, Professor Wataru Yamadera, Associate Professor Kazuya Ono, Associate Professor Ayumu Tateno, Assistant Professor Tatsuhiko Itoh, Assistant Professor Hiroshi Itoh, Professor Hisatsugu Miyata, Professor Kazutaka Nukariya, Associate Professor Motohiro Ozone, Associate Professor Rieko Shioji, Assistant Professor Minako Koga, Assistant Professor Satoshi Kawamura, 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 performed research in psychotherapy, psychopathology, and child psychiatry. Our child psychiatry group has investigated care systems for developmental disorders in the psychiatry unit. The treatment of children and adolescents with psychiatric disorders has been limited to a few specialized facilities in Japan. However, cases of psychiatric disorders among children have become common in general outpatient units. Thus, such cases should also be treated in general psychiatric inpatient units. However, the treatment of such cases requires particular strategies. Therefore, since 2000, we have been investigating various treatment strategies for 156 cases in general psychiatric inpatient units. On the basis of observations from this investigation, we have devised new treatment strategies for these cases in general psychiatric inpatient units.

We then began to study the attention problems of developmental disorders and psychotic disorders. This study found that the quality of attention was maintained more in autistic spectrum disorders than in schizophrenia but also found that when many tasks were added, the quality of attention tended to decrease. In the field of psychotherapy, we attempted to develop a prototype of dialectical behavior therapy for Japanese patients. The functional levels of patients with autism spectrum disorder (ASD) without intellectual disability (ID) are extremely diverse. Therefore, methods to implement simple psychotherapeutic approaches on an outpatient basis for ASD without ID are needed. We have, therefore, developed The Jikei Diary Training for ASD as a therapeutic approach for people who have ASD without ID.

Morita therapy group

In cooperation with psychotherapists of other schools for such treatments activities as cognitive-behavioral therapy and psychoanalysis, we have been developing programs and materials to train young psychiatrists to master the basic techniques of the clinical interview. We undertook practical research towards obsessive-compulsive disease with ASD.

We started practical research towards the application of Morita therapy to adolescent patients and patients with *hikikomori* (withdrawal). In addition, we continued the following studies: the psychopathology of social anxiety disorders, factors in the recovery of patients with depression through inpatient Morita therapy, and the application of Morita therapy in 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 a novel anticraving 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) effects of antipsychotics on dopamine transporter binding using positron emission tomography, 3) regulation of the salience network with antipsychotic agents via dopamine D2/3 receptors, 4) qualitative research on adherence in patients with schizophrenia, 5) the effect of modified electroconvulsive therapy on regulatory factors for gene expression, and 6) 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

Our studies included: 1) a study of changes in sleep structures and cognitive function with the menstrual cycle by means of the cyclic alternating pattern method, 2) empirical research regarding the efficacy of group cognitive behavioral therapy for primary insomnia and depression, 3) clinical research with the multiple sleep latency test of hypersomnias of central origin, and 4) an investigation of biomarkers of fatigue for obstructive sleep apnea syndrome.

Psychogeriatric group

We are performing several studies investigating the neural basis of neuropsychiatric symptoms and social function in patients with neurodegenerative disorders and other psychiatric disorders among elderly persons using neuropsychological evaluation and neuroimaging methods, such as brain magnetic resonance imaging and single-photon emission computed tomography. One study focused on the compensation mechanism for anosognosia in Alzheimer's disease (AD) and investigated the neural correlates of anosogonosia in AD. We found that anosognosia in AD could be correlated for with executive dysfunction and functional compensation through the semantic memory system. Another study focused on the effect of DNA methylation on the pathogenesis of AD. Our results suggest that DNA methylation in the non-SMC condensin II complex subunit H2 gene (NCAPH2)/lipase maturation factor 2 gene (LMF2) promoter region can be a useful biomarker for the diagnosis of AD and amnesic mild cognitive impairment, which is associated with hippocampal atrophy through apoptosis. We are planning to perform a longitudinal study and investigate further effects of these symptoms on the daily lives of patients and the burden on caregivers.

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 attempted an interpretation based on the Neojacksonism theory of Henri Ey about cases that presented with psychotic symptoms associated with epilepsy. Furthermore we reported changes in serum concentrations of antiepileptic drugs (especially new ones) 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. Satoshi Yoshimura to an annual conference to give a talk entitled "Understanding developmental disorders and mental disorders – approach through the Rorschach test." Then we discussed how to assess them. Furthermore, we trained graduate students of a clinical psychological course.

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Department of Pediatrics

Hiroyuki Ida, Professor Mitsuyoshi 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 ultimate aim of each subspecialty groups is to supply practical benefits to patients and their families through basic and translational research and clinical study.

Inherited metabolic disease group

Current therapies have a limited effect on bone involvement in patients with mucopolysaccharidosis type II (MPS II). We found that radiographic evaluations were extremely useful for monitoring bone involvement in patients with MPS II after gene therapy targeted at hematopoietic stem cell. Moreover, we found a chaperone effect of a certain disaccharide for some mutant enzymes from patients with MPS II. We are preparing for a gene therapy clinical trial for Fabry disease and MPS II under agreement with a company of biotechnology in US.

Neurology group

We are conducting basic research on several types of monogenic epilepsy by using patient-derived induced pluripotent stem cells and gene knockout rats to elucidate pathomechanisms and to establish innovative treatments, including cell therapy. We have refined our strategy for neuronal differentiation to obtain high-quality induced pluripotent stem cell-derived neurons, which are essential for replicating the "true" brain pathology *in vitro* and are to be used for cell therapy. Additionally, we have started a new project with magnetic resonance imaging to identify anatomical and cell biological characteristics of epileptogenesis in the developing brains of knockout rats. Furthermore, we have performed several clinical studies and identified the therapeutic efficacy of corticosteroids in epilepsy related to the protocadherin 19 gene (*PCDH19*) and the difficulty of diagnosing benign infantile seizures.

Nephrology group

We conducted a combined analysis of a nationwide survey for pediatric chronic kidney disease (CKD) and the National Report of Vital Statistics to investigate the effect of birth weight and gestational age on the risk of pediatric CKD. We found that both birth weight and gestational age are strongly associated with childhood-onset CKD.

We also identified possible risk factors for cyclosporine-induced nephropathy in idiopathic nephrotic syndrome and found that cyclosporine-induced nephropathy is associated with steroid-resistant nephrotic syndrome.

Infectious diseases and Immunologic Disorders group

We reported that gene therapy can provide a life-saving clinical benefit to patients who have X-linked chronic granulomatous disease (CGD) but lack a suitable donor. We also demonstrated that bowel inflammation in patients with CGD was improved by thalidomide without the progression of fungal or bacterial infections, suggesting that thalidomide might be an efficacious therapeutic option for patients with CGD colitis. Moreover, we showed that interleukin 1 β and basic fibroblast growth factor are important factors for the proliferation of human herpesvirus (HHV) 6 in an astrocyte cell line and that both were elevated in the cerebrospinal fluid of patients with HHV-6 encephalitis. Our results indicate that interleukin 1 β and basic fibroblast growth factor play key roles in the onset of HHV-6 encephalitis.

Hematology and Oncology group

We have performed several clinical studies for hematologic malignancies as a member of the Japan Child Cancer Study Group and the Tokyo Children's Cancer Study Group to explore novel therapies and diagnostic tools. We investigated late effects in survivors of childhood cancers, pediatric palliative care, and pain management in children with cancer. The mutation analysis by comprehensive Cancer Panel was performed to evaluate the molecular mechanism to develop malignancies associated with congenital anomaly syndrome. We demonstrated a novel mutation of the patched 1 gene (*PTCH1*) in medulloblastoma-associated Gorlin syndrome. Moreover, we have studied sequential urine polyamine in patients with retinoblastoma and reported that urine polyamine is a useful tumor marker for the recurrence of retinoblastoma.

Cardiology group

We evaluated the right ventricular remodeling in a mouse model of right ventricular pressure overload and the mechanism of angiogenesis in a rat model of the aortopulmonary collateral artery. Moreover, we produced a rat model of pulmonary hypertension caused by left heart disease. We have performed studies of the following topics: cardiac function and hepatic fibrosis in patients who have undergone the Fontan operation, correlation between stool calprotectin and protein-losing enteropathy, clinical assessment of infectious endocarditis, effectiveness of genetic analysis of ion channel disease, and the safety of congenital heart disease in the pediatric intensive care unit.

Allergy group

The main subjects of our research are as follows: (1) the roles of eosinophil, mast cells, and epithelial cells in the pathology of allergic diseases; (2) pediatric asthma; (3) food allergy; (4) atopic dermatitis; and (5) treatments for allergic diseases. We have organized and performed the following multicenter clinical studies: the ORIMA study (Effect of

Oral Immunotherapy in Preschool Children with Milk Allergy) and the DIFTO study (Daily versus Intermittent inhaled Fluticasone in TOddlers with recurrent wheezing), a multicenter, double-blind, randomized controlled study to investigate the effect of intermittent inhaled fluticasone in treating patients with infantile asthma. Recently, we have performed a study, which is, to our knowledge, the first in the world to investigate olfactory function in children with rhinitis.

Endocrinology group

We investigated the possible role of gonadotropin inhibitory hormone (GnIH) as a mediator between the hypothalamic-pituitary-gonadal axis and the hypothalamic-pituitary-thyroid axis involved in the regulation of puberty onset by thyroid status. We demonstrated that thyroid status alters GnIH messenger RNA expression *in vivo* and *in vitro* and that GnIH neurons express thyroid hormone receptors. These findings indicate that the effect of thyroid hormone on GnIH expression is mediated by thyroid hormone receptors. Moreover, we identified 3 novel mutations in 3 patients with suspected monocarboxylate transporter 8 deficiency and found new clinical aspects.

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Department of Dermatology

Hidemi Nakagawa, Professor Arihito Ota, Associate Professor Yoshinori Umezawa, Associate Professor Toshihiro Ito, Assistant Professor Keigo Ito, Assistant Professor Munenari Ito, Assistant Professor Takaoki Ishiji, Professor Akihiko Asahina, Associate Professor Masaaki Kawase, Associate Professor Yoshimasa Nobeyama, 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, and biologics, and topical therapies, such as vitamin D3 and corticosteroids, have been used, depending on disease severity and the degree to which quality of life (QOL) has been impaired in individual patients. Also phototherapy is effective and has been performed in the 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 interleukin 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 performing basic experiments with atopic model mice to investigate the mechanism of pruritus in atopic dermatitis. Clinical trials have been performed of topical phosphodiesterase-4 inhibitor and anti-interleukin 31 receptor antibody.

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 patients with cancers of an advanced stage, we perform multidisciplinary treatments, including immune checkpoint 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 MPNST in patients with neurofibromatosis 1 is approximately 10%. We have used the methylationspecific polymerase chain reaction (PCR) and real-time reverse transcriptase-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 1 and 2 and varicella-zoster virus are performed in this clinic. After the diagnosis is confirmed, suppressive therapies with varaciclovir 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., Stoken-church, UK), which contains tramadol hydrochloride and acetominophen.

Human papillomavirus infection

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

Contact dermatitis/drug eruption

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

Laser

The Q-switched ruby laser is useful for treating nevus Ota, acquired dermal melanocytosis, and ectopic Mongolian spot. On the other hand, nevus spilus is difficult to treat with the Q-switched ruby laser because it often recurs after 1 to 2 months. The recently introduced V-beam laser is effective for intractable vascular lesions. The ultra-pulse CO_2 laser can be used to quickly remove actinic keratosis, seborrheic keratosis, syringoma, and epidermal nevus.

Skin Care Clinic

Narrow-band ultraviolet B 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.

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Department of Radiology

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

Research Activities

1. Computed tomographic examination of eosinophilic chronic rhinosinusitis

Eosinophilic chronic rhinosinusitis is a newly recognized subtype of chronic rhinosinusitis which is characterized by peripheral blood eosinophilia and massive infiltration of eosinophils in the nasal mucosa. We proposed diagnostic criteria of eosinophilic chronic rhinosinusitis using computed tomography (CT) and analyzed its clinical usefulness.

2. Evaluation of the physiological laterality of the intracranial vein depiction with susceptibility-weighted imaging

The laterality of the intracranial venous system depicted with susceptibility-weighted imaging is an important index to suggest the presence of a related abnormality. However, because such laterality can also be seen in apparently healthy individuals, its significance is questionable. Therefore, we examined proven nonpathological cases in which the laterality of veins was depicted with susceptibility-weighted imaging.

3. The prevalence of apical cap on chest radiographs

The pulmonary apical cap (PAC) is pathologically identical to pleuroparenchymal fibroelastosis (PPFE), which is a new entity of chronic progressive interstitial lung disease. We evaluated the prevalence and temporal changes of PAC on radiographs of the chest and compared them to those of PPFE to assess the clinical significance of PAC and to determine the radiological borderline between PAC and PPFE.

4. Electrocardiography-gated multidetector CT for evaluating malformations of the coronary sinus

Evaluating malformations of the coronary sinus is important from the viewpoint of risk management for such procedures as inserting a central venous catheters or a pacing lead. We examined the diagnostic utility of electrocardiogram-gated multidetector CT in comparison with ultrasonic cardiography for evaluating coronary sinus abnormalities.

5. Use of Breast Imaging Reporting and Data System magnetic resonance imaging descriptors for differentiating mucinous carcinoma from fibroadenoma

Pure mucinous carcinomas (MCs) and mixed MCs similar to them both show findings similar to those of fibroadenomas, with myxomatous or edematous changes on both mammography and ultrasonography. Among breast carcinomas that have long T2 relaxation times, MCs are common and difficult to distinguish from fibroadenomas on T2-weighted images alone. We evaluated reliable Breast Imaging Reporting and Data System (BI-RADS) descriptors and non-BI-RADS magnetic resonance imaging (MRI) factors that contribute to differentiation of fibroadenomas from pure MCs or mixed MCs similar to

pure MCs.

6. MRI findings of primary cancers of the fallopian tube

We reviewed clinical, pathological, and imaging findings from 11 patients with primary cancers of the fallopian tubes. Such cancers have characteristic sausage-shaped tumors and are mainly solid, with a restricted diffusion and rim enhancement. For detecting and diagnosing these cancers, MRI, including diffusion-weighted MRI and dynamic contrast enhancement, is useful.

7. Evaluation of the diagnostic performance of tomosynthesis in pelvic insufficiency fractures

Digital tomosynthesis is a new digital technique that combines CT and digital image processing. Unlike conventional X-ray tomography, digital tomosynthesis is able to retroactively create an infinite number of arbitrary tomograms and is expected to be useful in musculoskeletal imaging. We evaluated the diagnostic value of digital tomosynthesis compared with radiography, CT, and MRI in pelvic insufficiency fractures.

8. Quantitative differentiation of benign and malignant mammographic circumscribed masses using intensity histograms

Through the use of mammography, circumscribed masses can be diagnosed with moderate accuracy using the intensity histogram standard deviation. Masses with a standard deviation less than the minimum of breast cancer can be roughly diagnosed as clinically benign. This study was published in the *Japanese Journal of Radiology*.

9. Comparison of cerebral perfusion and benzodiazepine receptor images between before and after adrenocorticotropic hormone therapy for infantile spasms

Adrenocorticotropic hormone therapy is effective for infantile spasms, but almost all patients show cerebral atrophy after therapy. The aim of this study was to determine changes of cerebral perfusion and benzodiazepine receptor images between before and after adrenocorticotropic hormone therapy.

10. Study comparing transarterial chemoembolization with cisplatin-eluting microspheres and bland embolization with microspheres after arterial infusion of cisplatin for multiple hepatocellular carcinomas in bilateral lobes

After commercial microspheres became available in Japan, we first treated multiple viable hepatocellular carcinomas on bilateral lobes by performing transarterial chemoembolization with microspheres (HepaSphere[®], Nippon Kayaku Co. Ltd., Tokyo, Japan) eluting a cisplatin material (IA-call[®], Nippon Kayaku) for arterial infusion. However, because the therapeutic effect was not sufficient, after the cisplatin material was infused, embolization was performed with nonchemical microspheres (Embosphere[®], Nippon Kayaku). Excellent therapeutic effects were observed and compared with these procedures about safety and feasibility.

11. Establishment and application of a general linear quadratic model that incorporates the time factor for the time-dose fractionation effect of radiotherapy

The linear quadratic model for quantitative evaluation has not been used to examine the lethal effect of irradiation but has been used to examine the biological effect of irradiation on normal tissue. However, a disadvantage in both cases is that the time factor is missing. Therefore, by using a general linear quadratic model that incorporates the time factor into the linear quadratic model, we studied the reproducibility of the time course of radiation

erythema of whole-breast irradiation during and after fractionated radiotherapy. (Grantin-Aid for Scientific Research (C), No. 23500369, final year)

12. Development of noninvasive quantitative evaluation of skin reaction due to breastcancer irradiation

Because skin reactions associated with radiotherapy are quantitatively evaluated, objective evaluation is difficult between observers. The purpose of this study was to verify whether changes in skin reactions associated with radiotherapy can be objectively evaluated with noninvasive quantitative methods. (Grant from the Japan Agency for Medical Research and Development)

13. Postoperative radiotherapy with volumetric modulated arc radiotherapy for prostate cancer

Three-dimensional conformal radiotherapy has long been used for the postoperative treatment of prostate cancer. However, we have not been able to achieve positive biochemical relapse-free survival. To improve results we have investigated volumetric modulated arc radiotherapy, which is a new type of treatment. With this treatment, toxicities of grades 3 to 5 have not occurred. In the future, we will report the present state of progression.

Publications

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Department of Surgery Division of Digestive Surgery

Katsuhiko Yanaga, Professor Norio Mitsumori, Professor Hideyuki Kashiwagi, Visiting Professor Masahiko Otsuka, Visiting Professor Tetsuji Fujita, Associate Professor Yoshio Ishibashi, Associate Professor Satoru Yanagisawa, Associate Professor Shuzo Kono, Assistant Professor Masaichi Ogawa, Assistant Professor Shuichi Fujioka, Assistant Professor Yasuno Futagawa, Assistant Professor Yasuro Futagawa, Assistant Professor Katsuhito Suwa, Assistant Professor Hiroaki Shiba, Assistant Professor Kazuhiko Yoshida, Professor Tomoyoshi Okamoto, Professor Nobuyoshi Hanyu, Visiting Professor Kenji Ikeuchi, Visiting Professor Takeyuki Misawa, Associate Professor Noburo Omura, Associate Professor Kouji Nakada, Associate Professor Hidejirou Kawahara, Associate Professor Yoshiyuki Hoya, Assistant 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

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.

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 the viability of the gastric tube with an intraoperative thermal imaging system during esophagectomy. The correlation between suitable graft construction and postoperative complications of a graft has been investigated. We also continue to

examine intraoperative recurrent nerve monitoring to prevent postoperative recurrent nerve palsies and to predict the degree of paralysis after surgery.

Limited surgery for gastric cancer may be aided by a search for sentinel lymph nodes, which are thought to be the first site of cancer cell metastasis. Lymphatic vessels and lymph nodes can easily be identified with an infrared endoscope. We were the first in the world to develop this procedure in 2001 and have used this technique for more than 300 patients with early gastric cancer. Regarding this procedure using an infrared endoscope, we have published 9 original or review articles in English. In addition, we have surveyed immunohistochemical staining and the expression of messenger RNA in tumor cells and evaluated the relationship between these expressions and clinicopathological findings. Such research revealed that zinc finger protein (ZNF) 217 is an independent prognostic factor for relapse-free survival and a novel prognostic biomarker in 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 patients have undergone gastrectomy, multiple tests of postoperative gastrointestinal function are performed to evaluate various gastrectomy procedures and to inform the patients of the appropriate management.

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 have started to register chemotherapy to develop a database. Together with the Department of Biochemistry we are committed to constructing 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. 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- κ B). In addition, NF- κ B is reported to directly induce MMP. The basement membrane is dissolved by MMP, and cancer cells enter the bloodstream and metastasize to another organ. Therefore, suppression of MMP might prevent metastasis after surgery. We will examine whether NF-kB decreases the recurrence and metastasis of colon cancer.

Hepatobiliary and pancreatic surgery

The outlines of our main research activities are as follows: 1) Living donor liver transplantation (LDLT) and regenerative medicine 2) Treatment for hepatocellular carcinoma (HCC) and control of recurrence

3) Chemotherapy for pancreatic and biliary cancer

4) Expansion of surgical indications for multiple hepatic tumors

5) Laparoscopic surgery for the liver, biliary tree, pancreas, and spleen

6) Navigation surgery for hepatobiliary and pancreatic diseases

7) Nutritional therapy for patients with cancer (enhanced recovery after surgery)

8) Control of surgical site infection

9) Effect of preoperative treatment of eltrombopag on splenectomy for idiopathic thrombocytopenic purpura

10) Molecular-targeting therapy for advanced HCC

11) Analyses of new biological tumor markers for HCC

The first LDLT was successfully performed for a patient with postnecrotic cirrhosis and HCC on February 9, 2007. Our first blood type ABO-incompatible LDLT (15th LDLT) was performed for a patient with primary biliary cirrhosis on June 5, 2015. Our 18th LDLT was performed for a patient with primary biliary cirrhosis on November 6, 2015. All 18 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 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. Also, we have started to apply molecularly targeted therapy to advanced HCC and to analyze new biological markers for HCC.

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

Toshiaki Morikawa, Professor Tadashi Akiba, Professor Shuji Sato, Assistant Professor Makoto Odaka, Assistant Professor Hiroshi Takeyama, Professor Satoki Kinoshita, Associate Professor Yasuo Toriumi, Associate Professor Kazumi Kawase, Assistant Professor Isao Tabei, Assistant 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 in progress.

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 who have such complications as advanced pulmonary emphysema, impaired pulmonary function, and extremely high age and 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 chest operations performed via thoracoscopy is more than 90%, which we assume to be the highest rate in the world.

The minimally invasive 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 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. The correlation of the detection of blood circulating tumor cells and the prognosis of patients with lung cancer is being examined.

The oncogenes of lung cancer are being analyzed with a next-generation sequencer.

A system for viewing videos on the Internet is now being developed and 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 feasibility, accuracy, and safety of this procedure in patients with breast cancer after neoadjuvant chemotherapy.

2) Evaluation of the usefulness of Sonazoid for detecting breast cancer

We performed phase II and III studies of the ultrasonographic imaging of the breast with the microbubble contrast medium Sonazoid (Daiichi Sankyo Co., Ltd., Tokyo) in collaboration with the Department of Radiology.

3) Evaluation of the effectiveness of exercise for psychiatric illness in patients after surgery for breast cancer

Many women experience a psychiatric illness, such as emotional distress, depression, and anxiety, after breast cancer is diagnosed. We have prospectively investigated the effects of exercise on psychological health.

4) Evaluation of the beneficial effect of cryotherapy for small cancers of the breast (Kashiwa Hospital)

5) Therapeutic strategy for oligometastatic breast cancer

We have analyzed patients with metastatic breast cancer for 30 years. The analysis indicates that oligometastatic breast cancer is a distinct subgroup with a long-term prognosis superior to that of metastatic breast cancer. We are performing prospective studies to better characterize oligometastatic breast cancer and to improve the prognosis of metastatic breast cancer.

2. Basic research

1) Development of breast cancer

We have used immunohistochemical techniques to investigate biological factors involved

in the progression of carcinoma in situ to invasive breast cancer.

2) Clinically useful biomarkers for triple negative breast cancer

Triple-negative breast cancer is a heterogeneous disease. We have investigated prognostic and predictive biomarkers for triple-negative breast cancer.

3) Circulating tumor cells and disseminated tumor cells

Circulating tumor cells in the peripheral blood and disseminated tumor cells in the bone marrow of patients with breast cancer are strong prognostic factors. We have investigated their clinical values for patients with early breast cancers.

4) Cancer stem cells

We evaluated how cancer stem cells differed in presence between primary breast tumors and metastatic tumors and how they were correlated with prognosis.

Endocrine

1. Clinical usefulness of JT-95

A monoclonal antibody, designated JT-95, was made against a thyroid papillary carcinoma obtained by our Department of Breast and Endocrine Surgery. In collaboration with the Division of Molecular Cell Biology of The Jikei University, we have investigated the clinical usefulness of JT-95.

In a clinical study accepted by the Institutional Review Board of The Jikei University, we are examining the antigen detected by JT-95 in the serum of patients with thyroid papillary carcinoma and of patients with breast tumors but without a thyroid mass.

2. Association between JT-95 and cell-to-cell inhibition

We found that the adhesion between cells was inhibited in proportion to the quantity of JT-95 added in vitro. We have investigated the mechanism of cell-to-cell inhibition and association with lymph nodes metastases.

3. The structure of antigen of JT-95

By using messenger RNA sequences and protein assays we are investigating the structure of the antigen detected by JT-95. This antigen contains a type of fibronectin. We are now detecting binding sites for JT-95 in the antigen.

Publications

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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 aneurysms, the treatment of peripheral arterial disease with new techniques.

Research Activities

Pediatric Surgery

1. Education

Education for medical students: Children undergoing surgery often have congenital anomalies. Lectures in pediatric surgery for students are based on embryology.

Education for training physicians: Three objectives for training physicians in pediatric surgery are: 1) learning how to obtain blood samples from pediatric patients, 2) understanding fluid therapy for pediatric patients, and 3) learning how to bury sutures.

Education for surgical residents: Residents are able to act as lead surgeons or assistants during pediatric surgery.

2. Clinical study

1) Minimally invasive and scarless surgeries

(1) Endoscopic treatment for vesicoureteral reflux using Deflux®

There are 3 options for managing or treating vesicoureteral reflux. We select treatment with Deflux[®] (Oceana Therapeutics, Ltd., Dublin, Ireland), an injectable dextranomer/ hyaluronic acid copolymer. Treatment was successful in 2 of 3 cases.

(2) Laparoscopic percutaneous extraperitoneal closure for inguinal hernia: The learning curve for attending surgeons and residents

Laparoscopic percutaneous extraperitoneal closure 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 means of a special suture needle (Lapa-Her-ClosureTM, Hakko Co., Ltd., Medical Device Division, Chikuma, Nagano, Japan). Concerns have been raised about the extensive learning curve for both attending surgeons

and residents to master this technique. This study assessed the difference in learning curves for the safe performance of laparoscopic percutaneous extraperitoneal closure by attending surgeons and residents.

(3) The Nuss procedure for treating pectus excavatum aims to force the sternum forward and hold it in place with an implanted steel bar without requiring a large incision to resect the abnormal cartilage. In this procedure, the curved steel bar is placed under the sternum through 2 small incisions on the sides of the chest. The number of patients with pectus excavatum treated surgically in our department is the third highest 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. Accumulating evidence indicates that cells communicate via the release and delivery of microRNAs packed into tumor-released exosomes. Understanding the role and mode of action of microRNAs from tumor-released exosomes is of paramount importance in the field of cancer biomarker discovery and for the development of new biomedical applications for cancer therapeutics.

A presentation entitled "A New Index for additional superior bar in Precuts Excavatum's Nuss Procedure" and authored by Shinsuke Ohashi, Shuichi Ashizuka, Joji Yoshizawa, Masashi Kurobe, and Takao Ohki was given at the 47th annual meeting of the Pacific Association of Pediatric Surgeons in Banff, Canada, in May 2014.

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 mortality 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. Although stent graft repair for TAAAs requires long operative and fluoroscopic times, this treatment is feasible and safe.

2. Development of endovascular repair of aortic arch aneurysms: Retrograde in-situ branched surgery and 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 through 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 that are commercially available in Europe for endovascular repair of aortic arch aneurysms after receiving approval from our institutional review board.

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. We participated in a randomized, controlled trial with a global registry; most patients were enrolled in the United States, but some were also enrolled in Germany and Japan. After the trial's 1-year primary endpoint was reviewed, the Zilver PTX received approval from the Japanese Pharmaceuticals and Medical Devices Agency in January 2012 and is now available in Japan.

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Department of Orthopaedic Surgery

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

General Summary

Basic Research

Our studies of bone metabolism and osteogenesis have been highly acclaimed both in Japan and abroad. The research on bone metabolism is focused on the relationship between osteoporosis and fracture risk. In basic research, we analyze hard tissue characteristics in a mouse model of mucopolysaccharidosis and, in cooperation with other specialty departments, are studying epigenetic modification in giant cell tumors. 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: shoulder joint, hand surgery, spine, hip joint, knee joint, foot surgery, trauma, osteoporosis, rheumatic diseases and sports. All teams maintain a high level of expertise and are actively involved in scientific activities.

The knee joint team has been performing total knee arthroplasty (TKA) with patientmatched instrumentation. They have analyzed effectiveness of cutting-edge technologies even more advanced than the surgical navigation system itself.

Research Activities

Results of arthroscopic Bankart repair for recurrent dislocation of the shoulder

Clinical results of arthroscopic Bankart repair for recurrent dislocation of the shoulder were reviewed. The subjects were 100 patients with a mean age of 30 years. The mean follow-up period was 4 years 10 months. Among all patients the postoperative recurrence rate was 13.6%. Risk factors for recurrence were sports activities involving collisions in teenagers, a large bony defect of the glenoid, and Hill-Sachs lesions. The arthroscopic Bankart procedure produced satisfactory mid-term results with almost the same rate of recurrence as in previous studies (8% to 19%). However, for patients with a high risk of recurrence, an additional procedure is necessary.

The current status of treatment provided by our hand-surgery team

Last year, an injectable clostridial collagenase (Xiaflex®, Dublin, Ireland) that digests

pathological palmar fascia was introduced in Japan for the treatment of Dupuytren's contracture and has also become available for patients at our institution. After this clostridial collagenase was introduced, fewer patients with Dupuytren's contracture have undergone operative pathological fascia resection. In addition to Dupuytren's contracture, hand diseases treated at our department include trauma cases, such as fractures, tendon ruptures, and neurovascular injuries; degenerative diseases; and tumors. We also use microsurgical techniques to repair nerves and tendons.

Reduction of spinal burst fractures with the monoaxial percutaneous pedicle screw system Anterior or anterior-posterior fusion surgery has often been performed for spinal burst fractures and has achieved good outcomes. Recently, the monoaxial percutaneous pedicle screw system without fusion has been shown to be less invasive and to better correct spinal deformities. The percutaneous pedicle screw without fusion often results in inadequate reduction or loss of correction. However, surgery with monoaxial screws leads to correction that is more satisfactory and longer lasting than is correction by surgery with fusion. Therefore, we suggest that this system be used to correct posttraumatic kyphotic deformities. The less-invasive monoaxial percutaneous pedicle screw system may offer an effective surgical option, even in elderly patients, and would enable early mobilization.

Short-term radiographic results of cementless press-fit cup implants: A comparative study between new generation highly porous metal-coated cups and conventional plasma spray-coated cups

The purpose of the present study was to compare early radiographic results between press-fit cementless cups with either a new-generation titanium coating (Regenerex[®], Zimmer Biomet) or a conventionally used plasma porous spray coating. A total of 103 hips implanted with titanium-coated cups and 103 hips implanted with plasma porous spray-coated cups were evaluated. The "initial gap" on postoperative radiographs was detected in 10 hips receiving titanium-coated cups and 4 hips receiving plasma porous spray-coated cups. Among these 14 cases, the initially observed gap was filled with newly formed bone in 8 cases with titanium-coated cups and 4 cases with plasma porous spray-coated cups.

Efficacy of patient-matched instruments and risk factors for postoperative delirium in TKA The results of this study showed that patient-matched instruments were effective for performing TKA. In regard to early rehabilitation, postoperative delirium has been shown to interfere with active postoperative mobilization. In addition to using patient-matched instruments, we examined potential factors related to postoperative delirium in patients after TKA. We found that low preoperative hemoglobin levels and a history of hypnotic usage were associated with postoperative delirium. We concluded that management of these factors might lead to better long-term results in patients undergoing TKA.

Evaluation of first ray mobility in patients with hallux valgus using weight-bearing computed tomography and a 3-dimensional analysis system

We compared the 3-dimensional mobility of first ray joints examined with computed

tomography between patients with hallux valgus and those with healthy feet. At the first tarsometatarsal (TMT) joint, the patients with hallux valgus showed significantly greater dorsiflexion, inversion, and adduction of the first metatarsal relative to the medial cuneiform, but loading of the foot caused major displacement at both the TMT joint and other joints. The hypermobility of the TMT joint has received much attention as a major cause of hallux valgus, but our results suggest that hypermobility extends across the entire first ray.

Poor bone quality in chronic obstructive pulmonary disease

Bone collagen cross-linking plays important roles in preserving bone strength. The quantitative and qualitative deterioration of lysyl oxidase and nonenzymatic cross-links of collagen (advanced glycation endproducts, pentosidine) might be affected by increased oxidative stress and glycation in patients with osteoporotic femoral neck fractures, diabetes, and chronic obstructive pulmonary disease.

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Department of Neurosurgery

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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 in the past year. Research in these areas is 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 patients with intracranial aneurysms have visited our department. As one of the world's leading aneurysm treatment centers, The Jikei University has placed a great value on establishing a precise real-time database of patients with aneurysms.

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

2. Analysis of biofluid mechanics on human intracranial aneurysms using a computational fluid dynamics

The main topics of our current studies include: 1) development of novel variables, 2) clarifying the relationship between hemodynamic patterns and the risk of rupture, and 3) development of dedicated software for computational fluid dynamics for angiography workstations.

3. Development of a novel intracranal stent device for the treatment of brain aneurysms A novel intracranial stent device for treating brain aneurysms is being developed.

A preclinical animal study is in progress. This project is supported by a research grant from 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 2016.

4. Establishment of a telemedicine network utilizing a novel software for smartphones Recently "Join," the next generation of telemedicine software, is available for any smartphone user. The application allows all medical staff to have instant access to the picture archiving and communication system in The Jikei University Hospital and allows the staff to communicate with an online bulletin board system. The application has been released in collaboration with NTT Docomo, which is Japan's largest mobile service provider, with more than 60 million customers.

Brain tumor

1. Immunotherapy against 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 antigens, this function is performed most efficiently by professional antigen-presenting cells, of which dendritic cells (DCs) are the most potent.

After exposure to tumor-associated antigens (TAAs), DCs process and express TAAderived epitopes in combination with MHC class I and II molecules on their cell surfaces and induce TAA-specific cytotoxic T-lymphocyte and T-helper type 1 subsets, respectively. We have previously shown that immunotherapy for glioma with fusions of DCs and glioma cells induces safe, tumor-specific immune responses. In a recent study, we observed that polyinosinic:polycytidylic acid (Poly[I:C]) transfection induced high levels of interleukin (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 T-helper type 1 response. At the 73_{rd} annual meeting of the Japan Neurosurgical Society and the 32_{st} annual meeting of the Japan Society for Neuro-Oncology.

2. Study of intraoperative imaging with C-arm CT-

We use a C-arm CT, *syngo* DynaCT system (Siemens Medical Systems), and an imageanalysis software program for metal-artifact reduction in the surgical resection of brain tumors. Intraoperative imaging with this system helps increase the resection rate of tumors with a surgical navigation system and photodynamic diagnosis with 5-aminolevulinic acid. The purpose of this study is to establish safe technical innovations for the surgical resection 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 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

The Division of Pediatric Neurosurgery performs operations for patients with spina bifida, myeloschisis, spinal lipoma, hydrocephalus caused by various medical conditions, cranial facial anomaly, and brain tumor and follows them up postoperatively at the outpatient clinic. In the last 10 years we have treated more than 1,700 new cases of various entities. We currently consist of a consultant, a division staff, and a resident and promote clinical research through various clinical activities.

For spina bifida, we are currently examining the prognosis of neurological functions by operating under neuromonitoring. We are also developing operative procedures for hydrocephalus using neuroendoscope and proposing the usage of navigation systems.

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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 the grafting of 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 was supposed to compromise the shape of the nasal pyramid. Recently we have been collaborating with otorhinolaryngology 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 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 under 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 resistance was less than the standard for adults, and the nostrils never collapsed under forced inspiration. Nasal valve compromise can cause nasal obstruction, even when the septum is straight, but can easily be treated with techniques well known 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 the joints. Use of the Ilizarov minifixator is an effective and lessinvasive 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 who 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%) and consisted 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 Yuzuru Nakamura, Professor Yoshimasa Sakamoto, Associate Professor Ryuichi Nagahori, Asssitant Professor Hirokuni Naganuma, Assistant Professor Yoshihiro Ko, Assistant Professor Kiyozo Morita, Professor Ko Bando, Professor Koji Nomura, Assistant 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 included those establishing excellent surgical performance, investigating new techniques, and evaluating alterations in cardiac performance and long-term results after cardiac surgery. New projects we started in the adult team are a risk analysis in adult cardiac surgeries by using national cardiac database and the development of evaluation methods in surgical skill performance for trainees. The experimental animal studies were performed to address clinical problems we are facing. A recent topic for adult surgery is the introduction of new fields: transcatheter aortic valve replacement and a left ventricular assist device program. We started with them by collaborating with other departments. We are also continuously performing several experimental studies with in-vivo models. The experimental projects include evaluating protection of the heart during cardiac arrest and the pulmonary valve. A project we started last year was visualization of the cardiac conduction system in human heart specimens by the high-resolution phase contrast computed tomographic imaging. The major activities are described below.

Research Activities

Echocardiographic evaluation of the postoperative coaptation geometry of the left atrioventricular valve in a complete atrioventricular septal defect

Postoperative echocardiographic assessment was performed in 18 patients, who had undergone complete repair of an atrioventricular septal defect, to analyze the function of the left atrioventricular valve.

Development of a new quantitative method for assessing pulmonary vascular volume with computed tomography

A new method was developed to measure the total pulmonary vascular volume, and its clinical role was evaluated in 38 patients.

Clinical investigation on myocardial protection during a pediatric heart surgery

In infants who had undergone open-heart surgery for a ventricular septal defect, an atrioventricular septal defect, or other congenital malformations with various cardioplegic strategies, biochemical markers for myocardial injury (troponin T) and oxidative stress

(8-iso-prostane) were evaluated.

Experimental studies of "remote preconditioning" as a new therapeutic strategy of myocardial protection

An experimental study in an *in-vivo* piglet model was performed to test the cardioprotective effects of "remote preconditioning" (intermittent ischemia/reperfusion of a remote organ before myocardial reperfusion).

Visualization of the cardiac conduction system in human heart specimens by the high-resolution phase contrast computed tomographic imaging

The visualization of the AV conduction axis within whole heart specimens was feasible with the use of PCCT and verified by subsequent histological examination. Nondestructive evaluation of the AV conduction axis in cardiac specimens and its 3-dimensional representation may allow more comprehensive examination of the conduction tissue in congenital heart anomalies.

Clinical study of adult cardiac surgery

1. Comparison of the early hemodynamic performance between conventional and newly designed bioprosthetic aortic valves

To compare the early hemodynamics of the third-generation high-performance Trifecta valve (St. Jude Medical, St. Paul, MN, USA) and the Magna Ease aortic valve with those of the Perimount aortic valve (Edwards Lifesciences Corp., Irvine, CA, USA), we retrospectively examined 266 patients who had aortic valve replacement, using 173 Perimount valves, 52 Trifecta valves, and 41 Magna Ease valves. The Perimount valves demonstrated a higher pressure gradient than did the Trifecta and Magna Ease valves. Severe patient-prosthesis mismatch (PPM: indexed effective orifice area (EOA) < 0.65 cm²/m²) was present at hospital discharge in 8.3% of patients with the Trifecta valve, 5.6% with the Magna Ease valve, and 21.4% with the Perimount valve. The new valves were associated with excellent early hemodynamic performance and provided lower gradients and larger EOA to reduce the rate of severe PPM.

2. Avoidance of PPM after mitral valve replacement with a new type of prosthesis

The effect of PPM after mitral valve replacement is still controversial. In this clinical report, we described 2 patients with small stenotic mitral valves. These patients have received a new type of mechanical prosthesis (ATS M36022mm, Century Medical, Inc., Tokyo, Japan), which has an EOA larger than that of a conventional prosthesis. The new mechanical valve for a small mitral annulus played an important role in avoiding PPM, as identified by an indexed EOA of less than 1.2 cm²/m².

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 i- m- a-, 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 \geq 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 (\geq 24 hours).

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Department of Obstetrics and Gynecology

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

Inactivating somatic mutations of genes that encode subunits of the switch/sucrose nonfermentable chromatin remodeling complex have attracted much interest in cancer cells. We performed the screening analysi with an inhibitor kit and cancer cells in which the AT-rich interactive domain 1A gene (*ARID1A*) was of the wild type or had been knocked out. The results identified the compounds as a potential therapeutic target for *ARID1A*-mutant cancers. To explore the interaction between *ARID1A*-mutant cancers and the compound, we will perform further assays.

2. A noninvasive diagnosis of ovarian clear cell carcinoma

The aim of this study was to establish a noninvasive diagnostic procedure for ovarian clear-cell carcinoma (CCC) by evaluating circulating tumor DNA and Pap smear-derived DNA from patients. We successfully demonstrated that our noninvasive digital polymerase chain reaction-based method of diagnosis can be used to detect gene amplification or mutation or both in circulating tumor DNA/Pap smear-derived DNA from ovarian CCC.

3. Interleukin 6 expression and its relationship with clinicopathological features including ARID1A expression in stage I CCC of the ovary

We aimed to identify the interleukin 6/ARID1A expression signature associated with patient characteristics in stage I CCC by means of immunohistochemical analyses. Interleukin 6 expression is likely a useful prognostic marker in stage I CCC.

4. Identification of the function of *FOXL2* mutation in granulosa cell tumor of the ovary We addressed a drug-screening method using the granulosa cell line inducible with the wild-type or mutant forkhead box L2 gene (*FOXL2*) to establish novel treatments specific for this mutation. We found that the transforming growth factor β pathway is involved in tumor growth.

5. MicroRNA gene expression signature driven by overexpression of microRNA-9 in ovarian CCC

This study aimed to elucidate potential clinical and biological associations of ovarian cancer-related microRNA gene expression profiles in ovarian high-grade serous carcinoma and CCC. In CCC, overexpression of microRNA-9 may affect pathogenesis by targeting E-cadherin.

Perinatology

1. Novel nonsense mutation in NLRP7 associated with recurrent hydatidiform mole

This study aimed to clarify the genetic and epigenetic features of recurrent hydatidiform mole in Japanese patients. This report is, to our knowledge, the first of a Japanese case of recurrent hydatidiform mole with a novel homozygous nonsense mutation of the NLR family pyrin domain containing 7 gene (*NLRP7*) and a specific loss of maternal DNA methylation of differentially methylated regions.

2. Increased expression of perforin, granzyme B, and C5b-9 in villitis of unknown etiology

Villitis of unknown etiology is associated with fetal growth restriction. This is, to our knowledge, the first report to assess villous injury, especially from the viewpoint of villous apoptosis, in a placenta with villitis of unknown etiology. An activated perforin/granzyme pathway and C5b-9 are suggested as possible mechanisms of apoptosis.

3. Antenatal treatment of myelomeningocele with 3-dimensional skin by using induced pluripotent stem cells

Myelomeningocele is a congenital abnormality resulting in exposure of the spinal cord to amniotic fluid. We obtained stem cells from amniotic fluid and induced differentiation into a skin lineage to create biomaterial. We developed a novel fetal therapy by using biomaterial that can be transplanted less invasively.

4. Single-cell DNA sequencing of fetal cells in maternal peripheral blood for noninvasive prenatal diagnosis

To develop a new risk-free method for obtaining precise genetic information from fetuses, we attempted to purify the fetal nucleated red blood cells in maternal peripheral blood and to develop the method for single-cell DNA sequencing with these fetal cells.

5. Exploring the unknown genetic causative candidate factors of recurrent abortions in Japanese women by using high-resolution whole-genome single-nucleotide polymorphism microarray analysis

To detect unknown genetic causative candidate factors of recurrent abortions in the Japanese, we performed high-resolution whole-genome single-nucleotide polymorphism microarray analysis in cases of recurrent abortion with no anatomical or medical causes in Japanese women and attempted to find genetic changes that are possible causative factors for recurrent abortions.

6. Noninvasive prenatal diagnosis of fetal sex and rhesus D status with circulating cell-free DNA

We attempted to develop a method to detect fetal sex and the rhesus D blood type with circulating cell-free DNA in maternal peripheral blood. Moreover, we assessed whether the method can be introduced as a clinical examination and whether the same protocol

can be adapted to prenatal examinations for other diseases and the phenotypes of fetuses.

Reproductive endocrinology

1. Investigation of the relationship between the optimal storage duration and the fertility of mouse ovary: Basic research for clinical application of ovarian transport

In Europe ovarian tissue is often transported overnight before being frozen, but the maximum storage time when ovaries are transported to increase fertility has no been investigated. This study aimed to clarify the relationship between the optimal storage duration and fertility. The study's results suggest that prolonging the ovarian storage time reduces fertility in mice. Thus, to minimize damage, ovaries should be frozen immediately after being harvested or should be transported as rapidly as possible.

2. Psychosocial care for oncofertility patients in Japan

Psychosocial care should be established for oncofertility patients who seek fertility preservation. We interviewed several healthcare providers working on oncofertility at Northwestern University (Evanston, IL, USA). A patient navigator is clearly recognized as the person for first contact from oncologists. A patient navigator provides patients with initial information about oncofertility. A psychologist cooperates closely with the patient navigator and provides psychological counseling to patients in a timely manner. We recognized that human resources, such as reproductive psychologists, cultivated by Japanese society for reproductive psychology, might play an important role. We examined the utility of decision trees for decision-making in Japan by using our data about oncofertility patients. The results showed that a social environment including the donation of oocytes or sperm, adoption, and other practices, should be prepared.

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Department of Urology

Shin Egawa, Professor Koichi Kishimoto, Professor Nozomu Furuta, Professor Yasuyuki Suzuki, Associate Professor Kenta Miki, Assistant Professor Takahiro Kimura, Assistant Professor Shoichi Onodera, Professor Hiroshi Kiyota, Professor Koji Asano, Professor Takashi Hatano, Assistant Professor Akira Furuta, 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 were 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) Adipose stem cell therapy for the treatment of interstitial cystitis, stress urinary incontinence, and underactive bladder

2. Clinical research: Several clinical studies have been performed 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 cancers

3) Comparison of cytokines, chemokines, and growth factors in patients with interstitial cystitis, overactive bladder, and chronic bacterial cystitis

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Department of Ophthalmology

Hiroshi Tsuneoka, Professor Hisato Gunji, Professor Satoshi Nakadomari, Associate Professor Tadashi Nakano, Associate Professor Tsutomu Sakai, Associate Professor Takuya Shiba, Assistant Professor Yoichiro Masuda, Assistant Professor Satoshi Goto, Assistant Professor Yasuhiro Ookuma, Assistant Professor Keigo Shikishima, Professor Genichiro Takahashi, Associate Professor Masaki Yoshida, Associate Professor Akira Watanabe, Associate Professor Takaaki Hayashi, Associate Professor Koichi Kumegawa, Assistant Professor Yoshiaki Kabata, Assistant Professor Tomoichirou Ogawa, Assistant Professor Tamaki Gekka, Assistant 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

We reported on a patient with recurrent refractory bilateral optic neuritis who twice tested negative for antibodies against aquaporin 4 before testing positive. Because seroconversion of these antibodies may occur at any time in patients with neuromyelitis optica spectrum disorders, this antibody should be measured several times if a patient has refractory optic neuritis with repeated episodes of recurrence.

Ocular oncology and histopathology

Optic nerve sheath meningiomas (ONSMs) are rare tumors that most often affect middleaged women. Recently, stereotactic radiotherapy has been recommended to treat patients with ONSM. We reviewed demographic data, frequency, clinical features, radiological imaging aspects, and the therapeutic strategy for ONSM.

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 screen-

ing 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 diseases

We have used 23-, 25- and 27-gauge transconjunctival vitrectomy system for macular hole, epiretinal membrane, macular edema, and rhegmatogenous retinal detachment. To evaluate the clinical efficacy of the single-piece intraocular lens (W-60[®], Santen Pharmaceutical Co., Ltd., Osaka, Japan) for combined pars plana vitrectomy, phacoemulsification, and intraocular lens implantation, we observed the visibility of the retina during vitrectomy and measured the depth of the anterior chamber preoperatively and postoperatively with an anterior eye segment tomogram (Pentacam[®], Oculus Optikgeräte GmbH, Wetzlar, Germany).

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 oph-thalmoscopy.

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 affibercept with subtenon triamcinolone acetonide injections for treating affibercept-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 affibercept.

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

Retinitis pigmentosa and related disorders have genetic heterogeneity. To identify pathogenic variants, we performed direct sequencing and whole-exome sequencing analysis of these disorders and successfully identified several novel pathogenic variants.

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.

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Department of Otorhinolaryngology

Hiromi Kojima, Professor Yutaka Yamamoto, Associate Professor Makoto Iida, Associate Professor Yoichi Seino, Assistant Professor Satoshi Chikazawa, Assistant Professor Tomomi Fukuda, Assistant Professor Nobuyoshi Otori, Professor Atsushi Hatano, Associate Professor Yoshinori Matsuwaki, Assistant Professor Daiya Asaka, Assistant Professor Jiro limura, Assistant Professor Takanori Hama, 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, skullbase 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

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Department of Anesthesiology

Shoichi Uezono, Professor Naohito Shimoyama, Professor Tsunehisa Tsubokawa, Professor Masanori Takinami, Associate Professor Chieko Fujiwara, Associate Professor Yasushi Mio, Associate Professor Shigehiko Uchino, Associate Professor Kazuhiro Shoji, Assistant Professor Keiko Kojima, Assistant Professor Yukino Kubota, Assistant Professor Takako Terui, Assistant Professor Sachiko Omi, Professor Shuya Kiyama, Professor Megumi Shimoyama, Associate Professor Masaki Kitahara, Associate Professor Ichiro Kondo, Associate Professor Akihiro Suzuki, Associate Professor Yoshie Taniguchi, Assistant Professor Yoichi Kase, Assistant Professor Gumi Hidano, Assistant Professor Hiroshi Sunaga, Assistant Professor Kotaro Kida, 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 2015 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 2015.

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 5 members of our faculty in 2015. 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 program for sonography. His expertise on this new technology will allow us to improve the quality of diagnostic procedures for acute care.

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 San Diego, California. 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 Mio's research has been focused on the effect of mitochondria on major organ preservation. He found protective effects of volatile anesthetic agents on mitochondria in renal cells. Doctor Shimoyama 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 Saito have been attempting to identify predictive factors affecting outcomes associated with acute kidney injury in patients immediately after cardiac surgery. 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

Kida K, Marutani E, Nguyen RK, Ichinose F. Inhaled hydrogen sulfide prevents neuropathic pain after peripheral nerve injury in mice. *Nitric Oxide.* 2015; **46:** 87-92.

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Yamakawa K, Howard-Quijano K¹, Zhou W¹, Rajendran PS¹, Yagishita D², Vaseghi M¹, Ajijola OA¹, Armour JA¹, Shivkumar K¹, Ardell J¹, Mahajan A¹ (¹UCLA, ²Tokyo Women's Hosp). Central vs. peripheral neuraxial sympathetic control of porcine ventricular electrophysiology. Am J Physiol Regul Integr Comp Physiol. 2016; **310**: R414-21.

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Heerdt PM¹, Sunaga H, Savarese JJ¹ ('Weill Med Coll Cornell Univ). Novel neuromuscular blocking drugs and antagonists. *Curr Opin Anaesthesiol.* 2015; **28**: 403–10.

Department of Rehabilitation Medicine

Masahiro Abo, Professor Kazushige Kobayashi, Professor Itaru Takehara, Associate Professor Kun Suk Chung, Assistant Professor Masanori Funakoshi, Assistant Professor Nobuyuki Sasaki, Assistant Professor Shu Watanabe, Professor Wataru Kakuda, Associate Professor Keiji Hashimoto, Associate Professor Hidekazu Sugawara, Assistant Professor Tadashi Suzuki, Assistant Professor Toru Takekawa, Assistant Professor

General Summary

The main research topics of our department are as follows: (1) repetitive transcranial magnetic stimulation (rTMS) for stroke, (2) dysphagia, (3) analysis based on a database, and (4) image analysis for brain injury.

Research Activities

Use of rTMS for treating stroke

1. Effects of low-frequency rTMS combined with intensive speech therapy on cerebral blood flow in poststroke aphasia

The results suggest the possibility that functional magnetic resonance imaging–guided low-frequency rTMS combined with intensive speech therapy may affect cerebral blood flow and contribute to the improvement of language function of patients with poststroke aphasia.

2. Role of brain-derived neurotrophic factor in beneficial effects of rTMS for upper-limb hemiparesis after stroke

The combination therapy of rehabilitation plus low-frequency rTMS seems to improve motor function in the affected limb, by activating the processing of brain-derived neurotrophic factor (BDNF). Both BDNF and its precursor, proBDNF, are potentially suitable biomarkers for poststroke motor recovery.

3. Therapeutic administration of atomoxetine combined with rTMS and occupational therapy for upper limb hemiparesis after stroke: a study of 3 patients

Our protocol of triple therapy of atomoxetine, low-frequency rTMS, and occupational therapy is safe and feasible for patients with upper-limb hemiparesis after stroke.

Dysphagia

1. Assessment of feeding and swallowing in children: Validity and reliability of the Ability for Basic Feeding and Swallowing Scale for Children

Significant correlations of the total Ability for Basic Feeding and Swallowing Scale for Children score, Fujishima's Grade of Feeding and Swallowing Ability, and the Functional Independence Measure for Children suggest the need for comprehensive assessments rather than assessments of individual feeding and swallowing functions.

2. Effect of tube feeding method on establishment of oral intake in patients with dysphagia after stroke: Comparison of intermittent tube feeding and nasogastric tube feeding The results of this study suggest that managing tube feeding by performing it intermittently achieves better outcomes for dysphagia rehabilitation than does nasogastric tube feeding.

3. A test of swallowing a citric acid solution is useful for screening for aspiration at the bedside and for detecting swallowing dysfunction at an early stage

The results suggest that a test of swallowing a citric acid solution is more sensitive for detecting dysphagia with laryngopharyngeal sensory dysfunction than is a modified water-swallowing test.

4. rTMS with intensive swallowing rehabilitation for poststroke dysphagia: An open-label case series

The 6-day in-hospital protocol of rTMS and intensive swallowing rehabilitation seems safe and feasible for patients with poststroke dysphagia and improves swallowing function.

5. Effect of dysphagia rehabilitation on oral intake in elderly patients with aspiration pneumonia

The data suggest that dysphagia rehabilitation has a positive effect on total oral intake in elderly patients with aspiration pneumonia and shows greater benefit if the pneumonia is mild rather than more severe.

Analysis based on database

1. Very early versus delayed rehabilitation of patients with acute ischemic stroke with intravenous recombinant tissue plasminogen activator: A nationwide retrospective cohort study

Patients with acute ischemic stroke undergoing very early rehabilitation after thrombolysis were more likely to achieve functional independence without an increase in adverse outcomes.

2. Effects of ankle-foot orthoses on functional recovery after stroke: A propensity score analysis based on a Japan rehabilitation database

These data suggest that the use of ankle-foot orthoses is a feasible option for improving the functional recovery of patients after stroke.

3. Clinical management provided by board-certificated physiatrists in early rehabilitation is a significant determinant of functional improvement in patients with an acute stroke: a retrospective analysis of a Japan rehabilitation database

These data demonstrated that clinical management provided by board-certificated physiatrists in early rehabilitation can lead to good functional recovery of patients with an acute stroke.

Image analysis for brain injury

1. Evaluation of regional white-matter volume reduction after diffuse axonal injury using voxel-based morphometry

The data showed that the volume of white matter decreased markedly in several regions of interest and correlated significantly with performance IQ and the processing speed index. The results indicate that this application can be used to evaluate diffuse axonal injury.

2. White matter structure and clinical characteristics of patients after stroke: A diffusion tensor magnetic resonance imaging study

Differences in generalized fractional anisotropy between hemispheres with lesions and hemispheres without lesions varied depending on the affected brain region, the patients' age at the onset of paralysis, and whether paralysis occurred in the dominant or nondominant hand.

Others

1. Trailblazing introduction of a hospitalization-associated disability prevention system using electronic medical recording systems at our hospital: Systemic attempt for forgotten complications in hospitalized patients

Our proposed hospitalization-associated disability prevention system using an electronic medical recording system appears to facilitate early rehabilitation and to prevent hospitalization-associated disability.

2. A reference value of higher brain function for resumption of driving in patients with brain injury

Results of a paper-based test serve as a guideline as to whether a patient is capable of resuming driving but do not represent an absolute standard. Therefore, the safety of resuming driving should be investigated on a case-by-case basis.

Publications

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feeding and swallowing scale for children (ABFS-C). *Brain Dev.* 2015; **37:** 508-14. **Sugawara H, Ishikawa M, Takayama M, Oka-**

Sugawara H, Ishikawa M, Iakayama M, Okamoto T, Sonoda S, Miyai I, Fujitani J, Tsubahara A. Effect of tube feeding method on establishment of oral intake in stroke patients with dysphagia:comparison of intermittent tube feeding and nasogastric tube feeding. Japanese Journal of Comprehensive Rehabilitation Science. 2015; 6: 1-5. **Kobayashi K, Kobayashi M, Abo M.** A citric-acid-solution swallowing test is useful as a screening test for aspiration at bedside and for the early detection of swallowing dysfunction. *J Clin Trials.* 2015; **5:** 245.

Momosaki R, Abo M, Watanabe S, Kakuda W, Yamada N, Kinoshita S. Repetitive peripheral magnetic stimulation with intensive swallowing rehabilitation for poststroke dysphagia: an openlabel case series. *Neuromodulation*. 2015; **18**: 630-4.

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nant of functional improvement in acute stroke patients: a retrospective analysis of Japan rehabilitation database. *J Stroke Cerebrovasc Dis.* 2015; **24:** 1019-24.

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axonal injury using voxel-based morphometry. *Magn Reson Med Sci.* 2015; **14:** 183-92.

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Department of Emergency Medicine

Takeki Ogawa, Professor Satoshi Takeda, Associate Professor Kenji Dohi, Associate Professor Taro Nameki, Assistant Professor Masahiko Uzura, Professor Kei Ohtani, Associate Professor Kenji Okuno, Associate Professor

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
- 6. Managing the course of Japan Advanced Trauma Evaluation and Care
- 7. Providing logistical support to the Japan Boxing Commission
- 8. Basic research on traumatic brain injury
- 9. Basic and clinical research on oxidative stress and emergency medicine
- 10. Advice to local authorities on plans for disaster medicine
- 11. Creation of the Disaster Medical Assistance Team deployment system
- 12. Management of the hospital emergency response drill, including Code Blue (Stat
- Call) and the Rapid Response System
- 13. Managing the Jikei Airway Management course for Patient safety (JAMP)

Research Activities

1. Supervision and development of ultrasound devices for the diagnosis and treatment of cerebrovascular disorders

- 2. Director of the 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. Publication of 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 on traumatic brain injury and oxidative stress
- 10. Basic research on heat stroke and neuronal injury

11. Development of therapy against free radicals in patients with acute neuronal conditions

12. Development of educational system in emergency medicine, including usage of simulation training

Publications

Saito O, Wang Z, Mitsumura H, Ogawa T, Iguchi Y, Yokoyama M. Substantial fluctuation of acoustic intensity transmittance through a bonephantom plate and its equalization by modulation of ultrasound frequency. *Ultrasonics.* 2015; **59**: 94-101.

Yamada S, Oikawa S, Komatsu T, Hirai T, Dohi K, Ogawa T. Early initiation of steroid pulse therapy for neuromyelitis optica in an emergency room setting. Acute Medicine & Sugery. 2016; **3:** 171-

3. Epub 2015 Aug 27.

Reviews and Books

Mitsumura H, Iwami T, Mitani Y, Takeda S, Takatsuki S. Aiming for zero deaths: prevention of sudden cardiac death in schools-statement from the AED Committee of the Japanese Circulation Society. *Circ J.* 2015; **79:** 1398-401.

Department of Laboratory Medicine

Tomokazu Matsuura, Professor Ken Kaito, Professor Hironari Sue, Professor Midori Kouno, Assistant Professor Yoshihiro Mezaki, Assistant Professor Akihiro Ohnishi, Professor Hiroshi Yoshida, Professor Kenichi Sugimoto, Associate Professor Setuko Akizuki, Assistant Professor

General Summary

The main studies were accomplished by 2 large-scale projects: the Research on the Innovative Development, provided by the Ministry of Health, Labour and Welfare of Japan, and the Practical Application of New Drugs for Hepatitis B, provided by the Japan Agency for Medical Research and Development. We performed studies to connect experimental medicine with clinical medicine.

Research Activities

Clinical microbiology

1. Several clinically isolated, previously unidentified bacterial strains were identified though gene sequencing of polymerase chain reaction-amplified 16S ribosomal RNA. We attempted to characterize the differentiation of methicillin-resistant *Staphylococcus aureus* at the strain level using whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry. We observed the congruence of mass spectrometry-based clustering and pulsed-field gel electrophoresis (established typing method) clustering.

2. We developed cell lines that produced hepatitis B virus in episomal for evaluating the antiviral action of new compounds.

3. We studied the biofilm formation ability of *Staphylococcus epidermidis*.

Clinical chemistry

1. We accumulated the case for studies, including patients with diabetes mellitus and diabetic complications. In addition, we measured serum pentosidine concentrations and endogenous secretory receptors for advanced glycosylation endproducts (AGEs). Hypoglycemic treatment reduced GA levels, glycation sites of albumin, and AGE levels with or without complications of diabetes mellitus. Keeping GA levels low would prevent the formation of AGE and may prevent the progression of diabetic complications.

2. We published an article about the performance of the latest version of our high-performance liquid chromatography lipoprotein cholesterol measurement system, which has been covered by health insurance since July 2013. A study to determine the high-density lipoprotein function of cholesterol efflux with stable isotope and to assess the risk of atherosclerosis has been supported by a research grant from the Ministry of Education, Culture, Sports, Science and Technology, and the fundamental method is currently being established. The collaborative study results of statin-related adverse events for glucose 3. We developed the fasting ¹³C-octanoic acid breath test as a technique to noninvasively evaluate the β -oxidation of the liver. ¹³C-octanoic acid is absorbed from the jejunum and is transferred to the liver via the portal vein. In the liver ¹³C-octanoic acid passes through the mitochondrial outer membrane without contribution of carnitine and is oxidized by β -oxidation. We can evaluate the beta-oxidation of the liver if we measure ¹³CO₂/¹²CO₂ in exhaling gas.

Clinical oncology

Ruxolitinib successfully decreased the burden of the mutant V617F allele of the Janus kinase 2 gene (*JAK2*). The burden of the *JAK2*V617F* was measured in patients treated with ruxolitinib, an inhibitor of tyrosine kinase subtypes JAK1 and JAK2. In a patient with continuous ruxolitinib treatment, the mutant allele burden was initially 72.4% but then decreased through 59.2% and 53.7% to 52.9%. However, in a patient who had stopped receiving ruxolitinib, no change was observed. A cytogenetic response with a decrease in the burden of mutant allele *JAK2*V617F* was observed after treatment with ruxolitinib.

Clinical psychiatry

We attempted interpretation based on Neojacksonism (from French psychiatrist Henri Ey) about the patients who presented the psychotic symptoms associated with epilepsy. Furthermore, we reported changes in serum concentrations of antiepileptic drugs (especially new ones) while patients with epilepsy were pregnant. We performed a study about preventing depression from recurring in patients with epilepsy. We examined the safety and efficacy of psychotropic drugs in several forms of psychosis associated with epilepsy.

Clinical physiology

In an examination in phases of menstruation we performed a basic examination of the walk analyzer.

Clinical immunology

A main theme of our research is the study of nonspecific reactions in the inspection of immunity. This year, we studied the nonspecific reactions in the measurement of SCC and the syphilis *Treponema pallidum* antibody.

Clinical cell biology and pathology

1. Hepatic stellate cells store vitamin A as a form of retinylester through an enzymatic activity of lecithin:retinol acyltransferase. The upregulation of the retinoic acid receptor α gene (*RARA*) at the posttranscriptional level and the subsequent upregulation of the cellular retinol-binding protein I gene (*CRBP1*) at the transcriptional level make a feedback loop toward the recovery of vitamin A-containing lipid droplets within the activated

hepatic stellate cells.

2. Using human liver biopsy specimens, we examined the stainability of transforming growth factor- β latency-associated protein of transforming growth factor- β degradates, which was a fibrogenesis marker of nonalcoholic steatohepatits/nonalcoholic fatty liver disease.

Publications

Shrestha R^{1,2}, Tatsukawa H³, Shrestha R^{1,4}, Ishibashi N⁵, Matsuura T, Kagechika H², Kose S¹, Hitomi K³, Imamoto N¹, Kojima S^{1,2,4} (¹*RIKEN*, ²Tokyo Med Dent Univ, ³Nagoya Univ, ⁴Tokyo Inst Tech, ⁵KOWA Company Ltd). Molecular mechanism by which acyclic retinoid induces nuclear localization of transglutaminase 2 in human hepatocellular carcinoma cells. *Cell Death Dis.* 2015; **6**: e2002.

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Kishimoto Y, Yoshida H, Kondo K. Potential anti-atherosclerotic properties of astaxanthin. *Mar Drugs.* 2016; **14:** E35.

Department of Endoscopy

Kazuki Sumiyama, Professor Hiroshi Arakawa, Assistant Professor Hirobumi Toyoizumi, Assistant Professor Hiroo Imazu, Associate Professor Keiichi Ikeda, 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 (\times 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 NBImagnifying 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 EUSguided 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.

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Department of Infection Control

Seiji Hori, Professor Hiroshi Takeda, Assistant Professor Yasushi Nakazawa, Assistant Professor Masaki Yoshida, Associate Professor Koji Yoshikawa, Assistant Professor Tetsuya Horino, Assistant Professor

General Summery

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, and outbreak and infection control.

Research Activities

Analysis for pathogenic factors of methicillin-resistant Staphylococcus aureus isolated by a blood culture

In cases of bloodstream infection caused by *S. aureus*, severe metastatic infections, such as infective endocarditis, septic pulmonary embolism, and iliopsoas abscess, may occur and lead to a patient's death. This trend is more common in methicillin-resistant *S. aureus* (MRSA) bacteremia than in methicillin-sensitive *S. aureus* (MSSA) bacteremia. Previously we revealed that in MSSA bacteremia, predictive factors for metastatic infection were a delay in appropriate antimicrobial treatment of > 48 hours, persistent fever for > 72 hours after starting antibiotic treatment, and lowest C-reactive protein levels of > 3 mg/dL for 2 weeks after the onset of bacteremia, we performed a broth microdilution method for the antibiotic susceptibility testing of 23 isolates that were clinically isolated during 2013. All 23 isolates were susceptible to vancomycin, teicoplanin, linezolid, daptomycin, and arbekacin according to the Clinical Laboratory and Standards Institute criteria, which indicated that the appropriate antibiotics were selected. We are now investigating the host-related predictive factors and MRSA-related pathogenic factors.

Clinical studies of combination antimicrobial therapy against multidrug-resistant Pseudomonas aeruginosa *with the Break-point Checkerboard Plate method*

We studied the effects of combination antibiotic therapy with the Break-point Checkerboard Plate method against clinical isolates obtained at our hospital from 33 cases of multiple-drug resistant *P. aeruginosa* bacteremia. Two combination therapies — colistin plus aztreonam, and amikacin plus aztreonam — showed synergistic effects. Two cases of multiple-drug resistant *P. aeruginosa* bacteremia were successfully treated with the combination antimicrobial therapy of amikacin plus aztreonam.

Meta-analysis of urinary tract infections in patients receiving a sodium-dependent glucose co-transporter 2 inhibitor

The aim of this meta-analysis study was to determine the relationship between sodiumdependent glucose co-transporter 2 (SGLT2) inhibitors and urinary tract infections in patients with diabetes mellitus. Of 27 studies reviewed, only 9 showed that patients receiving an SGLT2 inhibitor had an increased rate of urinary tract infections. On the other hand, none of these studies showed an increased rate of severe renal infections, such as pyelonephritis or urosepsis. In addition, in many studies that evaluated the efficacy of SGLT2 inhibitors, the definition, classification, and additional risk factors of urinary tract infections were not described.

Revisiting a method for diagnosing toxoplasmosis: Development of the Toxoplasma *Kill-ing Observation test*

Toxoplasma gondii, the most successful protozoan, infects approximately one-third of persons worldwide. In most people who are infected, except for pregnant women and immunocompromised patients, toxoplasmosis is a self-limited disease with mild symptoms or no symptoms. Immunocompromised patients, such as those who have acquired immunodeficiency syndrome, have undergone organ transplantation, or use steroids, are at risk for *Toxoplasma* encephalitis, pneumonitis, and retinitis. Most of these cases are caused by relapses and flares from the bradyzoite, a slowly duplicating form of *Toxoplasma* which is dormant in host tissues. On the other hand, primary infection during pregnancy is a risk factor for congenital toxoplasmosis, which causes intrauterine growth retardation, hydrocephaly, mental growth retardation, retinitis, and even fetal death.

Many types of serodiagnosis are widely used to detect toxoplasmosis around the world. However, because immunoglobulin (Ig) G and IgM are the only antibodies against *Toxoplasma* available for serodiagnosis in Japan, diagnosis can be complicated. If toxoplasmosis has relapsed in patients, IgM is usually negative, and even if IgG is positive the present infection cannot be discriminated from previous infections. The diagnosis of congenital toxoplasmosis with IgG and IgM is another issue. Some studies have found that IgM remains over the threshold for more than 2 years and that the positive predictive value of IgM is only 45.98%. This kinetics of IgM has a risk of leading to misdiagnosis.

The Sabin-Feldman dye test has been used since 1948. The dye test uses the serum of a subject to evaluate the aggregate ability of a tachyzoite-cidal immunoglobulin titer. The classic serodiagnosis test still has high sensitivity and specificity as confirmed diagnosis. A problem with the dye test is the complicated evaluation method, in which stained tachyzoites must be counted under visual recognition. We examined this problem with a tachyzoite expressing green fluorescent protein, which is an alternative marker for evaluating the deactivation of the tachyzoite. The new, improved dye test, the *Toxoplasma* Killing Observation test, has the advantages of objectivity and retention for evaluation and provides outcomes equivalent to those of the classic Sabin-Feldman dye test.

Effect of infection control team round against Gram-negative bacilli bacteremia at Kashiwa Hospital

At Kashiwa Hospital, the infection control team (ICT) makes rounds for patients with positive blood cultures and has an intervention for the proper use of antibiotics. We examined the effects of the ICT's rounds against Gram-negative rod (GNR) bacteremia in the hospital. In the 6 months from August 31 to March 1, 2015, we investigated the cases in which GNR had been detected in blood cultures. After drug-susceptibility testing, the

attending physician changed the antibiotic to one with a narrow-range spectrum (de-escalation) in about half of the patients with GNR bacteremia. De-escalation could be promoted by the ICT rounds. After ICT intervention, the use of antibiotics was appropriate. In the future, cooperation is needed between the ICT and attending physicians.

Drug-resistant bacteria have become a major social problem

We performed a questionnaire without filling in the hospital name in 750 Japanese teaching hospitals to investigate the present conditions of infection control in Japan. The status of basic infection control measures, such as the hand hygiene and antimicrobial stewardship, was included in the questionnaire. We received answers from 369 hospitals (49.2%). Questions about MRSA isolation in 2013 were answered by 286 hospitals and produced a mean isolation rate of 46.8%. Questions about the consumption of alcoholic hand disinfectant by an entire ward, except the operating room, were answered in 2013 by 248 hospitals, leading to a median consumption rate of 6.0 ml/patient-day. Therefore, in Japan, the basic infection control measures should be quickly improved. Especially needed is the improvement of hand hygiene.

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Department of Dentistry

Katsuhiko Hayashi, Professor Shigeru Suzuki, Associate Professor Akihiko Ikai, Professor

General Summary

- 1. Clinical studies of temporomandibular disorders (TMDs)
- 2. Anatomical studies of the temporomandibular joint (TMJ) of marsupials
- 3. Clinical studies of perioperative oral management

Research Activities

Clinical studies of TMDs

1. Effects of personal-computer operating guidelines on the prevalence of TMDs at general clinical offices in metropolitan Tokyo

We developed a test for screening for TMDs (2007). In a previous study, we found a significant relationship between the prevalence of TMDs and personal-computer operating time in female subjects. Following this study, we gave guidelines concerning visual display terminal (VDT) operation to members of the Tokyo Dental Association who did dental checkups. These members then taught the guidelines to patients who underwent dental checkups. We performed a study to examine the effects of the VDT operation guidelines on the prevalence of TMDs among subjects who participated in dental checkups of female office workers in the Tokyo metropolitan area. We classified the subjects into 2 groups: 79 subjects who were patients before the guidelines had been developed (from 2007) and 223 subjects to whom the guidelines were presented (56 subjects from 2012 and 167 subjects from 2013). Between these groups we examined differences in the effects of VDT operating time on the prevalence of TMD. The personal-computer operating times were significantly greater in guideline-presented subjects (mean, 8.0 hours; 25th and 75th percentiles, 6.0 and 8.0 hours) than in pre-guideline subjects (mean, 2.0 hours; 25th and 75th percentiles, 0.0 hour and 5.0 hours). The results suggest that that VDT guidance is useful for decreasing the risk of TMDs and that research on contributing factors for TMDs should be continued.

2. TMJ dislocation observed in children

The mandibular condyle loses its normal relative position and becomes dislocated. The condition often occurs in elderly patients and rarely occurs in children. However, TMJ dislocation occurs in children most often because of falls or other types of trauma. In many patients, such as patients who present with incomplete dislocation, the dislocation naturally repositions when the mouth is held widely open for crying. For this reason, encouraging the child to cry to induce reduction may be an effective treatment method with manipulative reduction. Ensuring compliance in children with limited mouth opening is difficult, and immobilization devices might affect jaw growth; therefore, long-term follow up of mandibular development and morphology is important.

Anatomical studies of the TMJ of marsupials

1. Morphological characteristics of the TMJ in the pouch young of the Tasmanian devil We recently reported the absence of the articular disk, which is a constant structure in mammals, in the TMJ of the adult Tasmanian devil. However, whether the articular disk disappears when the animal grows was unknown. The aim of this study was to determine with histological examination whether the articular disk is present in the TMJ of the pouch young animal. The TMJ of the fresh cadaver of a pouch young animal, whose crown-rump length was 43 mm, was examined with microscopy and microcomputed tomography. The pouch young TMJ was morphologically confirmed to lack an articular disk. This finding suggests that the articular disk of the Tasmanian devil is naturally absent. The high-cell-density fibrous tissue on the condylar surface apparently does not differentiate into the articular disk but develops into the thick mature fibrous layer covering the condyle which was previously observed in the adult Tasmanian devil.

Clinical studies of perioperative oral management

1. Intervention with perioperative oral management

Perioperative oral management became covered by insurance with the revision of medical fees in 2012. On the basis of our previous results, our department has adopted measures to increase the rate and advance the period of dental service use. The subjects for the study were 605 patients who underwent surgery for malignant tumors in the craniocervical region, respiratory or digestive system, or other parts of the body under general anesthesia, cardiac surgery, organ or bone marrow transplantation, chemotherapy, or radiotherapy within the 1-year period from April 2014 through March 2015. The referring department, the primary disease and its treatment, and the period between the initial consultation and primary disease treatment were compared with data obtained during April 2012 through March 2014. Furthermore, adverse events in cases of oral or hematogenous infection were statistically examined. The number of patients treated in our department had increased yearly, by 311 from the 2012 fiscal year. Adverse events were observed more frequently among patients undergoing chemotherapy and patients with reduced immunity. Perioperative oral management before treatment of the primary disease was not possible in 26.4% (28 of 106) of patients but was performed in a significantly lower percentage of patients (7.2%, 36 of 499 patients) (p < 0.01). Preoperative dental intervention was effective for preventing complications in patients targeted for perioperative oral management, particularly those with an increased risk of reduced immunity. Thus, pretreatment dental intervention might be important for patients undergoing chemotherapy or steroid therapy.

2. Perioperative oral function management

We examined the performance of perioperative oral function management. The subjects for the study were patients for whom perioperative oral function management was requested in the Department of Dentistry of Daisan Hospital during the period from January 2012 to May 2015. Most of the requests were related to chemotherapy or radiotherapy. Perioperative oral function management led to shorten stays in the hospital and the intensive care unit and to fewer cases of postoperative aspiration pneumonia and ventilator-associated pneumonia.

Publications

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Department of Transfusion Medicine

Tetsunori Tasaki, Professor

General Summary

1. In 2013, a study group funded by a Health and Labor Science Research Grant was formed to develop guidelines for easily distinguishing between transfusion-related acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO). We (principal investigator Tasaki and colleagues) have summarized the 3-year study on the adverse respiratory events associated with blood transfusions. We hope that the new guidelines will be useful for performing the correct differential diagnosis of the signs and symptoms associated with TRALI and TACO, so that the appropriate therapy can be implemented.

2. We attempted to demonstrate that antileukocyte antibodies in donor blood products directly induce a respiratory disorder in the transfusion recipient. However, we did not find a significant association between adverse events and antileukocyte donor antibodies.

3. Two years have passed since we began our participation in a study group investigating the relationship between hematopoietic stem cell transfusion and adverse reactions. The investigation of adverse reactions in hematopoietic stem cell infusions is an ongoing prospective multicenter observational study. To date, the incidence of adverse reactions is almost 40%, based on data collected from 522 patients. A detailed analysis will be performed after data is collected from a total of 1,000 patients.

4. In 2015, we participated in 2 multicenter collaborative studies: (1) Evaluation of Mononuclear Cells Positive for CD34 Marker by Fixed RBC Method and (2) A Study of Appropriate Demand and Use of Blood Products by Information and Communication Technology.

5. The summaries of 2 studies performed by our department were presented at the annual meeting of the Japan Society of Transfusion Medicine and Cell Therapy. One presentation was entitled "The significance of using aliquots of blood products in pediatric transfusion" and involved a study that found that the importance of using aliquots of units of packed red blood cells for pediatric surgeries could not be evaluated, because all the aliquots of a unit were used during surgery. However, the transfusion of aliquots was useful for children in pediatric wards. Unnecessary transfusions and waste were avoided. The second presentation was entitled "Current situation and problems of harvesting peripheral blood stem cells from related donors in our hospital." The safe harvest of stem cells from healthy allogeneic donors is a top priority. Assessing CD34 cell counts in harvested mononuclear cells the day before apheresis might prevent the unnecessary administration of granulocyte colony-stimulating factor and lighten the burden of donors.

Research Activities

1. Although a worldwide standard, which was developed in 2004 in Toronto, Canada, is

commonly used to diagnose TRALI, no such standard exists for diagnosing TACO. In 2013, a study group was formed to perform a study to establish Guidelines for the Differential Diagnosis of the Signs and Symptoms Associated with TRALI and TACO and for the Treatment of TRALI and TACO, Considered to be Serious Problems of Blood Transfusion. The final guidelines were presented in the *Journal of the Japan Society of Transfusion Medicine and Cell Therapy* (46[3], 2016).

2. Antileukocyte antibodies are thought to be a cause of TRALI. Because the rate of positive findings is higher in female donors, especially parous women, male donor-derived plasma has mainly been used for transfusion, with a subsequent reduction in transfusionrelated dyspnea. However, few prospective studies have confirmed the association between antileukocyte antibodies in blood products and respiratory disorders in transfusion recipients. Our data from 70 patients who received allogeneic platelet units containing antileukocyte antibodies also could not confirm a significant association between antileukocyte antibodies in blood products and respiratory disorders in transfusion recipients. Because of the lack of evidence and the low incidence of TRALI, we have concluded that preventing TRALI by transfusing male-derived plasma should be strongly recommended with a continued focus on the relationship between TRALI and antileukocyte antibodies. Our philosophy is similar to that used to prevent posttransfusion graft-versus-host disease, which is to irradiate units of allogeneic blood instead of focusing on assessing the similarities of the HLA profiles of blood donors and recipients.

3. Various adverse reactions, including nausea and fever, occur in patients who undergo hematopoietic stem cell transplantation (HSCT) and are probably due to the simultaneous transfusion of anticoagulant and cryoprotectant (dimethylsulfoxide). However, little information is available for preventing such reactions after HSCT. In 2015, Dr. Ohto (Fukushima Medical University) formed a study group of 20 investigators which began gathering detailed information on HSCT: the timing, duration, types, and severity of adverse events and the necessity of therapy. We provided data on 40 patients who underwent HSCT before December 31, 2015. The interim results indicate that adverse reactions occurred in a significantly higher percentage of patients undergoing allogeneic bone marrow transplantation (51.7%) than of patients undergoing autologous peripheral blood stem cell transplantation, (40.3%). Among the various adverse reactions, a change in blood pressure was the most significant. The target number of patients is 1,000.

4. CD34 is thought to be a surface marker of HSCs, and accurately quantifying HSCs in samples of mononuclear cells is important for HSCT. The principal investigator, Dr. Okuyama, and his colleagues (Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital) sent 2 different cell samples to 55 HSCT facilities for CD34 cell quantification. The results were collected and compared to the results measured with the gold-standard method. Each facility was graded; we received a grade of A for each sample.

5. Although allogeneic blood donation is safe, we have a responsibility to use blood products carefully and appropriately, especially for children with long life expectancies. From a practical perspective, the division of a unit of packed red cells (approximately 160 ml) into 2 to 3 aliquots seems sensible, because the amount of blood used at one time is small. However, because the shelf life of platelets is only 24 hours in Japan, the division of platelet units into aliquots is not practical.

The 2013 approval in Japan of stem cells harvested from unrelated donors will increase the number of donors who will choose peripheral blood stem cell harvest.

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

Functional and structural damages after eccentric contraction in mouse skeletal muscle Modestly intense eccentric contraction (ECC) increases muscle mass, but strenuous ECC damages muscle. To optimize the ECC protocol for muscle growth, we examined contractility and structures of skeletal muscle after ECC. Tibialis anterior muscle in adult male mice underwent a protocol of isometric contraction, ECC, or passive stretch. Contraction was elicited by supramaximal electrical stimulation via the sciatic nerve in situ. Each protocol comprised 3 sets of 30 tetani or stretches or both every 10 seconds with 5-minute intervals between the sets. Contractility at optimal muscle length and electron microscopic structure was examined after the protocol. Several initial muscle lengths (1 and 0.9 optimal muscle lengths) and stretch extents (0.05, 0.1 and 0.2 optimal muscle lengths; at 2.0 optimal muscle length/second) were tested. Passive stretch caused no functional changes. Isometric contraction reduced the maximum force and contraction time of twitch more prominently at longer muscle lengths. The ECC prominently reduced maximum twitch/tetanus force and twitch contraction time and prolonged tetanus contraction. Extra force developed during ECC beyond optimal muscle length seemed crucial for the functional deteriorating effects. Microscopic observation suggested that ECC beyond optimal muscle length caused failures in excitation coupling, cross-bridge formation, and the integrity of sarcomere to induce the functional deteriorations. An ECC beyond optimal muscle length should be avoided in training protocols for muscle growth.

Mechanism underlying development of fatty liver

The pathology of fatty liver due to a high-fat diet is largely unclear. We investigated fat metabolism in rats with a fatty liver resulting from consumption of a high-fat, low-carbo-hydrate (HFLC) diet without an increase of total caloric intake. Four-week-old male Sprague-Dawley rats were randomly assigned to the control and HFLC groups, and rats were fed the corresponding diets *ad libitum*. Food intake per body weight was significantly lower in the HFLC group than in the control group. Thus, body weight was similar in both groups. Fat in the liver prominently accumulated in the HFLC group and was accompanied by suppression of *de novo* lipogenesis in the liver and elevation of the blood leptin level. In addition, electron microscopic observation revealed many lipid droplets accumulated within the hepatocyte and revealed a reduction in mitochondria content in

the liver derived from rats in the HFLC group. Our findings confirm that fatty liver occurred following consumption of a HFLC diet, even without increased caloric intake. Furthermore, *de novo* lipogenesis is not likely to be a crucial factor to induce fatty liver. Instead, ultrastructural abnormalities in mitochondria may decrease β -oxidation and contribute to the development of fatty liver.

Effects of chronic exercise on the exocrine and endocrine pancreas in WBN/Kob-Fatty rats

Chronic pancreatitis is an inflammatory disorder, causing a progressive and irreversible dysfunction of the exocrine and endocrine pancreas. Recently, the leptin receptor-deficient WBN/Kob-Fatty rat was developed as a model of chronic pancreatitis and diabetes with obesity. The purpose of this study was to investigate whether chronic exercise and a restricted diet increase the decreased pancreatic function of WBN/Kob-Fatty rats. Male WBN/Kob-Fatty rats (age, 6 weeks) were divided into fatty-obese (n = 10), fatty-diet restriction (n = 8), and fatty-exercise (n = 9) groups. Nonobese rats were used as a control (n = 6). The control and fatty-obese rats had free access to food, and the fatty-diet restriction rats and the fatty-exercise rats had food intake restricted to 69% and 70% of the fatty-obese level. The fatty-exercise rats voluntarily ran on a rotary wheel ergometer for 6 weeks. Mean body weight and serum levels of glucose, amylase, and triglyceride were higher in the fatty-obese rats than in other groups. Pancreatic weight and protein content in the fatty-obese rats and fatty-diet restriction rats were lower than in the control rats and fatty-exercise rats. Insulin resistance in the fatty-obese rats was the worst of all the groups. Microscope observations of pancreatic tissue revealed abnormal endocrine areas and some inflammations in the fatty-obese rats. Normal spherical islets of Langerhans were observed in the control rats and the fatty-exercise rats. The interleukin 6 level of the pancreas in the fatty-obese rats was higher than in other groups. Twelve-week-old male WBN/Kob-Fatty rats had symptoms of pancreatitis and diabetes, but nonobese rats did not. These results indicated that obesity is a major factor to aggravate pancreatitis and diabetes. Chronic exercise with a restricted diet beneficially affected the exocrine and endocrine pancreas in WBN/Kob-Fatty rats through the treatment of obesity. The decreased pancreatic function in chronic pancreatitis and diabetes might be increased by an adequate exercise habit and a restricted diet.

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Department of Cell Physiology Division of Aerospace Medicine

Susumu Minamisawa, Professor

Masamichi Sudoh, 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 3 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.

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.

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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

Neuropathology of human immunodeficiency virus-related brain disease

Objective: This study investigated the neuropathology of human immunodeficiency virus (HIV)-related brain disease.

Material and methods: We analyzed 8 cases of HIV-related brain disease. Formalin-fixed, paraffin-embedded tissues were cut for histopathological assessment. Brain, spinal cord, and peripheral nerves were stained with hematoxylin and eosin, Krüver-Barrera, Bodian, Zeel Nielsen, Gram, periodic acid-Schiff, and Grocotte stains, and immunohistochemical examination was performed with primary antibodies against glial fibrillary acidic protein, neurofilaments, myelin basic protein, amyloid precursor protein, CD68, ionized calcium-binding adapter molecule 1, CD3, CD4, CD8, CD20, CD80, human simplex viruses 1 and 2, varicella zoster virus, human herpesvirus 6, measles, Epstein-Barr virus, histoplasma, John Cunningham virus agnoprotein, John Cunningham virus viral protein 1.

Results: We observed HIV encephalopathy in 6 cases, HIV leukoencephalopathy in 3 cases, and vacuolar myelopathy in 1 case. No cases showed neuronal loss in the cerebral cortex. Microglial activation and amyloid precursor protein-immunopositive axons were observed in 6 cases. Invasion of CD8 cells without CD4 cells was observed in the cerebral cortex in 1 case clinically diagnosed as immune reconstitution inflammatory syndrome. As HIV-related infections, progressive multifocal leukoencephalopathy, cytomegalovirus infection, Cryptococcus infection, and tuberculosis were revealed.

Discussion: From the mid-1990s, pathological findings of HIV-related brain disease changed and became complicated with the introduction of combination antiretroviral therapy. One disease is central nervous system- immune reconstitution inflammatory syndrome, defined as unexpected worsening of the neurological condition and consistent with inflammation mediated by activated CD8 T-cells. We should evaluate HIV-related brain disease with the reference of clinical information.

Autopsy of the first patient with mucopolysaccharidosis type I, Hurler-Scheie syndrome in Japan to be treated with enzyme replacement therapy

Hurler-Scheie syndrome (HSS) is a rare autosomal disorder caused by deficiency of α -L-

iduronidase, a lysosomal enzyme that hydrolyzes the terminal α -L-iduronic acid residues of dermatan sulfate and heparin sulfate. Recently, patients with HSS have effectively been treated with enzyme replacement therapy (ERT). We reported the autopsy of a 42-yearold woman with HSS who received the first treatment with ERT in Japan. The HSS had been diagnosed when the patient was 2 years old and had been treated with ERT when the patient was 30 years old. Brain weight was 875 g. Atrophy of the cerebral cortex and dilatation of the sulcus were observed. Myelopathy existed from Th4 to Th10. Ballooned neurons in the central and peripheral nervous systems were stained with Alcian blue and immunostained with antibodies against subunit c mitochondria ATP synthase. With electron microscopic examination, membranous cytoplasmic inclusions and Zebra bodies were observed in the nervous system, hepatocytes, vascular endocytes, and chondroxytes. Secondary tauopathy was observed in the limbic system and the central gray matter of the midbrain.

Administrative autopsy of a man with spinocerebellar ataxia type 31

We reported the administrative autopsy of a 64-year-old man with spinocerebellar ataxia type 31 diagnosed by histopathological findings and molecular biological analysis. Spinocerebellar ataxia type 31 is an autosomal dominant form of late-onset purely cerebellar ataxia caused by a complex pentanucleotide repeat containing (TGGAA)_n in an intronic region shared by 2 genes: the brain expressed, associated with NEDD4 gene (BEAN) and the thymidine kinase 2 gene (TK2). With neuropathological examination, cerebellar Purkinje cells are preferentially affected with nuclear deformity and reduced in number and are often surrounded by halolike amorphous materials.

Publications

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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 lives and young athletes engaged in school sports clubs or dedicated to training within sports clubs. In 2015 we have been mostly focused on basic research.

Research Activities

Arthroscopic surgery for posterior ankle impingement syndrome

We investigated results of arthroscopic surgery for posterior ankle impingement syndrome in 9 patients (10 feet) and evaluated pathological features of the condition in regard to various sport activities. Among 6 female ballet dancers examined, all presented preoperatively with crepitus when moving their toes in a plantar position. Operative findings showed markedly thickened tendon sheath of the flexor hallucis longus. The mean time until a return to sport activities was 2 months; however, ballet dancers returned after a mean time of 5 months to recover the *en pointe* position. Arthroscopic surgery for posterior ankle impingement syndrome is less invasive and has advantages, such as direct intraoperative understanding of pathology dynamics, that are difficult to achieve from imaging data.

Correlation between magnetic resonance imaging findings and time before a return to sport activities because of disabled throwing shoulders

Magnetic resonance imaging (MRI) findings of 58 patients with disabled throwing shoulders were examined and evaluated for possible correlation with physical findings and the time before a return to play. Of the patients, 16 had normal MRI findings, 23 had bone lesions, 20 had labrum tears, 12 had tendon lesions, and 5 had subacromial manifestations on MRI scans. Separation of the epiphysis in the proximal humerus was observed in only junior high school students, and slant appearance was found in junior and high school students. We did not find any correlation between MRI findings and physical findings or the time before a return to play. We concluded that in patients with disabled throwing shoulders, MRI findings do not always reflect the underlying pathology.

Comparison of 3 self-stretch techniques for tightness of the 2_{nd} internal rotation in patients with disabled throwing shoulders

We compared the effectiveness of 3 types of self-stretch exercises — the all-fours posterior stretch (APS), the cross-body stretch (CBS), and the internal rotation stretch (IRS) —

in 48 baseball pitchers with disabled throwing shoulders. The inability ratios due to pain were as follows: 4% with APS, 23% with CB, and 42% with IRS. The range of motion of the 2_{nd} internal rotation before stretch exercises did not differ significantly among the groups. However, the ranges of motion obtained just after corrective stretching in the subjects who did APS and CB were significantly higher than that in subjects who did IRS. We concluded that APS is the most useful stretching technique for persons with disabled throwing shoulders; it can be performed painlessly and can effectively improve the 2_{nd} rotational range of motion.

Changes in nerve-muscle coordination caused by muscle fatigue: An analysis using the silent periods of the quadriceps and hamstrings

We investigated changes in the silent periods of the quadriceps and hamstrings before and after inducing muscle fatigue in 12 healthy adults. Fatigue of the quadriceps and hamstrings was induced by using an isokinetic dynamometer (Cybex International, Medway, MA, USA). We did not find any significant differences in premotion time between loaded and nonloaded sides or before or after the muscle load on the same side. However, the switched silent period measured after the physical load application was significantly longer than that measured before load application on both sides. This study indicates that muscle fatigue delays nerve-muscle coordination on the sides to which loads have been or have not been applied.

Difference in muscle strength of the quadriceps and hamstrings between contact and noncontact injuries: A comparison analysis before and after reconstruction of the anterior cruciate ligament

Preoperative and postoperative muscle strength of quadriceps and hamstrings were measured in patients who underwent reconstruction surgery of the anterior cruciate ligament. The ratio of the affected to the nonaffected side, the ratio of quadriceps and hamstrings, and the weight-bearing index were compared between 14 patients with a contact injury and 13 patients with a noncontact injury. Hamstrings were lower on the affected side than on the nonaffected side, and the hamstrings-to-quadriceps strength ratio in patients with noncontact injuries was lower than that in patients with contact injuries. The results suggest that patients who have had noncontact injuries should begin hamstring strength exercises soon after undergoing reconstruction of the anterior cruciate ligament.

A case of dislocated talar neck fracture after injury during a soccer game

We reported the rare case of an 18-year-old male soccer player who had received injury leading to a dislocated fracture of the talar neck during a soccer game. The fracture was diagnosed as type II by the Hawkins classification and was manually reduced. Bone union was obtained by conservative treatment, and complications were not observed 1.5 years after the injury.

A case of gluteus minor muscle strain developed in a female marathon runner

We reported on the rare case of a 39-year-old female marathon runner who presented with strain of the gluteus minimus muscle following an injury. A high signal-intensity

region on short-tau inversion recovery MRI scans was found across the entire volume of the right gluteus minimus muscle. Overuse syndrome due to long-distance running was considered to be the underlying pathomechanism of this injury.

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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 Earth-quake 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) is in progress with more than 400 patients with CKD IIIa, b and the publication is on going.

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.

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, while the prospective study is fixed cases and the publication is ongoing.

Check-up and evaluation

Research regarding the onset and development of hyperuricemia and CKD is ongoing. The analysis of the FEATHER study will be completed in March 2016, and a manuscript is being prepared. 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.

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Department of Innovative Interventional Endoscopy Research

Hisao Tajiri, Professor

Masato Mitsunaga, Assistant Professor

General Summary

This department was established in April 2015, aiming at new methods of endoscopic diagnosis and treatment and the development of apparatuses, with the purpose of supporting and teaching the standardization of endoscopic medicine in domestic and foreign facilities.

Research Activity

Endoscopic submucosal dissection (ESD), which was developed in Japan, has been followed by various improvements to conduct examinations safely, promptly, and accurately. Subsequently, new minimally invasive endoscopic treatments, such as endoscopic fullthickness resection and endoscopic treatment applying robotic technology, are being developed. As Japan's society ages, less-burdensome, minimally invasive endoscopic treatments significantly contribute to patients. While social demand for endoscopic medicine is growing, new methods should be used for endoscopic treatment and the development of instruments.

This department plays a role in developing educational structures for endoscopy for physicians in Japan, other Asian countries, Russia, the Middle East, and South America.

Development of supporting devices for ESD and clinical evaluation

Recently, there has been a shift from open surgery to laparoscopic surgery, a minimally invasive method to reduce the patients' burden reduces and increase quality of life. In addition, for early gastrointestinal cancers, oral and transanal endoscopic therapy is being performed more often with flexible endoscopes. However, a problem of oral and transanal endoscopic therapies, such as ESD, is that they are safely performed by only skilled physicians. Existing surgical instruments are electric scalpels, which pierce the forceps at an entrance 2.8 mm in diameter to penetrate a flexible endoscope, which moves only to-andfro. Lifting affected parts and cutting open the inside while a fiberscope is operated are extremely difficult with all these devices. The endoscopic system, which can be operated by a surgeon's freely moving right and left hands, has long been expected, and bending, elastic forceps have been researched and developed all over the world. However, these forceps are not practical because the smallest ones are still 4 mm in diameter and cannot be inserted in the forceps channel (2.8 mm) of the existing elasticity endoscope. An article specially made to order is expensive. Because present flexible endoscopes are expensive, efforts must be made to develop ones that can be used economically on a daily basis. Because the forceps's outlet of commercially available flexible endoscope is 3 mm in diameter, a flexible forceps 2.6 mm in diameter must be inserted. Hashizume et al. have

succeeded in the trial manufacture of the world's first flexible flexure forceps 2.6 mm in diameter (Nakadate R, et al. Endoscopy 2015; 47(9): 820-4). This medical device might achieve practical economic use. Furthermore, to increase utility and decrease costs, 2 control sticks are equipped to the fixed base so it can be manipulated stably, and the grip of a flexible scope and its console are placed so that they can be reached and conducted at the same time by a single endoscopist. The flexible endoscopes can be removed any time manual manipulation is necessary. Because they are robotic devices not requiring motors, they are close to their practical application. We organized "A study group for ESD supporting devices" in 2015 and have been repeating animal studies of *in vivo, ex vivo*, for technology development so that ESD for the stomach, esophagus, and colon can be conducted with this device without stress.

Endoscopic optical molecular imaging for cancer

Molecularly targeted therapies, such as monoclonal antibodies, have recently been widely used to treat various types of cancer and have improved outcomes. The use of molecularly targeted medicine for patients with cancer generally depends on the level of molecular expression in the targeted tumor; therefore, a method of companion diagnosis must be developed when a molecularly targeted therapy is developed. Histological testing and genetic testing are usually used for companion diagnosis; however, the necessary time can delay the start of an appropriate therapy. We have developed methods of molecular target-specific fluorescence cancer imaging by using cancer-specific monoclonal antibodies and optical fluorescence probes for real-time companion diagnosis. In this study, we attempted to develop a method for optical molecular imaging of cancer with a laserequipped endoscope system (LASEREO; FujiFilm Corp., Kanagawa, Japan) and molecular targeted-fluorescence probes which enables us to diagnose molecular expression in a real-time manner. We examined in vitro detectability of fluorescence probes, Alexa Fluor488-conjugated trastuzumab, fluorescein, and a cresyl violet solution. To improve signal detectability, the endoscope system was modified from the default settings by changing the output of laser units (410 and 450 nm) and image-processing algorisms. We were able to see the signals of AF488-trastuzumab and fluorescein with the naked eye; however, contrary to our expectations, none of 3 probes were able to detect through the endoscope system. In the case of cresyl violet, the excitation laser was not suitable for signal detection. In summary, we need to optimize the image processing of the endoscope system to detect fluorescence signals for further studies.

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Department of Innovation for Medical Information Technology

Hiroyuki Takao, Associate Professor and Director

General Summary

This course deals broadly with information and communication technology (ICT), an area that has recently seen remarkable developments, 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 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, intrahospital 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 software program called "Join," which is to be covered by insurance in Japan. The research investigates such factors as the cost-effectiveness of communication in the treatment of patients with stroke, in which the time before diagnosis and treatment is especially important.

Research and development of a health support application

We are researching and developing a software program called "MySOS." During an emergency, this application seeks help from nearby people and helps decide whether trips should be made to a hospital by referring to emergency manuals for adults and children. Future development will focus on enabling linkage with hospitals.

Internet of Things development

We are going forward with the development of Internet of Things wearable devices as a means of accumulating large quantities of data. In the development of blood-pressure meters in smartphones or in devices resembling wristwatchs and band-type electroencephalograms, we are advancing development from the standpoint of storing large amounts of personal medical information into a vast Internet database ("the cloud") via smartphones and defending against illness.

Mobile phone electromagnetic wave effects

We are doing research on how smartphones affect medical equipment. The research will determine whether issues are related to the use of smartphones at medical care facilities. We are publishing a paper on this subject.

Medical equipment development

We are discussing the development of medical equipment and 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 performing various studies of the introduction of ICT medical care. The use of ICT in various aspects of nursing and caregiving might improve work efficiency in these areas. The aim is to put this into practice.

Medical results of using robots

We are conducting research, using the human-shaped robot Pepper (SoftBank Corp., Tokyo, Japan), on the interaction between robots and people. We are studying what changes occur in health care facilities when people see and come into contact with robots.

Research Center for Medical Sciences Division of Gene Therapy

Toya Ohashi, Professor and Director

Hiroshi Kobayashi, Associate Professor

Research Activities

As we have done for many years, we have been developing novel therapeutic methods to overcome limitation of current therapy for lysosomal storage disease (LSD) and intractable cancer, which has no therapy or only inefficient therapy. Our missions are finding new seeds for such novel therapy and translating new seeds to clinical use.

Induction of immune tolerance in enzyme replacement therapy for LSDs

We have already shown that immune reactions against enzymes in enzyme replacement therapy for LSDs have a negative effect on its efficacy. This year, we found that an anti–B-lymphocyte stimulator antibody, which reacts with B cells and is approved in the United States, induced tolerance against enzymes in enzyme replacement therapy for Fabry disease.

Recombinant lentiviral vector and zinc finger nuclease–mediated gene therapy for LSDs We investigated the bone system in mucopolysaccharisosis type II (MSP II), and detected increasing of bone mass, trabecular bone, bone density, and bone strength comparing to the normal. We are preparing to investigate changes in these factors by the intervention of gene therapy.

Developing human hematopoietic cells transplantable MSP II mouse model

We are developing hematopoietic stem cell targeted gene therapy for MSP II. To translate this methodology to clinical use, we are developing a human hematopoietic cells transplantable humanized MSP II mouse model. The NOG mice were developed in the Central Institute for Experimental Animals (Kawasaki, Japan) and shows marked engraftment of human hematopoietic stem cells. This year, we knocked out the iduronate 2-sulfatase (IDS) gene (*IDS*) in fertilized eggs from NOG mice by using gene-editing technology (CRISPR/Cas9) and transferred embryos to the uteri of NOG mice. We are now analyzing newborn mice.

Development of novel therapy for Pompe disease by using a proteasome inhibitor

We previously demonstrated that the proteasome inhibitor bortezomib can improve acid α -glucosidase (GAA) activity in patient fibroblasts and cell lines transiently expressing several types of mutants of the GAA gene (*GAA*) mutants. However, the efficacy of bortezomib treatment in an animal model is still unclear. In this study, we generated a missense mouse model of Pompe disease expressing human GAA with M519V amino-acid substitution and analyzed the efficacy of bortezomib treatment on GAA activity in our novel Pompe mice. Bortezomib treatment increased levels of precursor and mature GAA in the hearts of M519V mice but not in skeletal muscle. Increased GAA activity was also observed in the heart but not in skeletal muscle. These results indicate that bortezomib can work as enzyme-enhancement molecule in an animal model and in cultured cell lines.

Effect of sulfated disaccharides on IDS in MSP II

Pharmacological chaperone therapy has attracted considerable attention as a potential treatment for LSDs accompanying central nervous system lesions. However, a pharmacological chaperone therapy for MSP II has not been developed. In this study, we focused on the sulfated disaccharides derived from heparin, which is a substrate of IDS, and evaluated the efficacy of oligosaccharides as pharmacological chaperones for MSP II. When sulfated disaccharides were incubated with recombinant IDS, thermal degeneration of the enzyme was significantly prevented. In addition, sulfated disaccharides increased IDS activity in fibroblasts from a patients with MSP II. These results suggest that sulfated disaccharides are candidate molecules for pharmacological chaperone therapy for MSP II.

Treatment for Fabry peripheral neuropathy with adeno-associated virus vector in murine Fabry disease model

Fabry disease is an X-linked LSD caused by mutation of the α -galactosidase A (GLA) gene (*GLA*), resulting in deficient activity of GLA. Peripheral neuropathy is a significant symptom of Fabry disease. We treated a murine model of Fabry disease with the human *GLA* gene encoded with an adeno-associated virus (AAV) serotype AAV rh 10 vector. The activity of GLA was increased in the heart, liver, spleen central nervous system and the peripheral nervous system. The murine model of Fabry disease showed dysesthesia compared with wild-type mouse. With the von Frey up-down test, dysesthesia was marginally more improved in AAV-treated mice than in untreated mice with Fabry disease. Our data indicates that gene therapy with AAV rh10 encoding the human *GLA* gene could be a new therapeutic approach for Fabry peripheral neuropathy.

Antitumor effect of nafamostat mesilate for digestive cancer and treatment of cancer pain We have previously reported that nafamostat mesilate, a serine-protease inhibitor, inhibits the activation of nuclear factor kappa B (NF- κ B) and induces the apoptosis of pancreatic cancer. Moreover, we have shown that the addition of nafamostat mesilate promotes apoptosis induced by gemcitabine or paclitaxel owing to the inhibition of the NF- κ B activation of pancreatic, gastric, and gallbladder cancers. The clinical usefulness of the combination of gemcitabine and nafamostat mesilate for patients with unresectable pancreatic cancer was examined in a phase II study. Because the standard therapies for unresectable pancreatic cancer are gemcitabine/S-1 and gemcitabine/nab-paclitaxel, we have investigated combination therapy with these anticancer agents and an NF- κ B inhibitor. Moreover, we have investigated the antitumor effect of combination therapy with new NF- κ B inhibitors, such as pomalidomide and recombinant thrombomodulin, for pancreatic cancer.

Ionizing radiation enhances epithelial-mesenchymal transition (EMT) and cancer metastasis. Neoadjuvant chemoradiation for colorectal cancer also enhances EMT. Therefore, we have suppressed EMT by inhibiting NF-κB or signal transducer and activator of transcription 3 in chemoradiation for colorectal cancer.

Cancer pain worsens the quality of life of patients with unresectable pancreatic cancer. We have shown the mechanism of cancer pain and investigated a new treatment strategy.

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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 research is to develop and establish novel cancer therapies. Concepts for new anticancer therapies, generated from the unique ideas of our researches, would be verified by basic and clinical studies so that they could be applied clinically. Most of our research has been on antitumor immunity.

Research Activities

Investigation of immunogenic mutated antigens in glioblastoma multiforme

Although the overall survival and progression-free survival of patients with glioma have significantly improved with dendritic/tumor cell vaccine therapy, a predictive marker of efficacy is unknown. Whole-exome and whole-transcriptome analyses were performed of patients treated with vaccine therapy to clarify the relationships of mutation-derived antigens and the gene expression profile to the efficacy of vaccine therapy. Mutated protein sequences obtained from detected variants by whole-exome analysis were predicted for binding affinity to human leukocyte antigen (HLA). Mutated peptides predicted as strong binders of HLA were selected as candidates for mutation-derived antigens. Approximately 40 candidates of mutation-derived antigens were detected in all cases. Some candidates were common in all cases. However, no candidates of mutation-derived peptides were found to be common in patients in whom vaccine therapy was effective. Expression of major histocompatibility complex (MHC) class II molecules in patients was higher when vaccine therapy was effective than when it was ineffective. These results suggest that the effectiveness of dendritic/tumor cell vaccine therapy in patients with glioma is related to MHC class II molecule expression rather than to the number of mutationderived antigens or specific mutated-derived antigens.

Nafamostat mesilate suppresses interferon γ *–inducible programmed cell death ligand-1 up-regulation in cancer cells*

The binding of programmed cell death ligand 1 (PD-L1) on tumor cells to programmed cell death 1 (PD-1) on activated T lymphocytes leads to inactivation of T lymphocytes, resulting in the immune escape of tumor cells. Because PD-L1 up-regulation is induced by interferon (IFN) γ secreted from activated cytotoxic T lymphocytes (CTLs), suppression of IFN γ -inducible PD-L1 up-regulation is important for CTL-mediated antitumor activity. We have found that nafamostat mesilate (NM), a serine protease inhibitor, potently suppresses IFN γ -inducible PD-L1 up-regulation in cancer cells. HLA class I is an essential molecule for T cell response and up-regulated by IFN-gamma just like

PD-L1. Interestingly, NM did not suppress IFN γ -inducible HLA class I up-regulation, and activation of signal transducer and activator of transcription 1/IFN regulatory factor 1 pathway was not affected by NM treatment. These results suggest that NM has a unique mechanism for PD-L1 suppression different from that of inhibition of signal transducer and activator of transcription 1 signaling.

Encryption of agonistic motifs for Toll-like receptor 4 into artificial antigens augmented the maturation of antigen-presenting cells

Adjuvants are indispensable for achieving a sufficient immune response from vaccinations. From a functional viewpoint, adjuvants are classified into 2 categories: "physical adjuvants," which increase the efficacy of antigen presentation by antigen-presenting cells (APC), and "signal adjuvants," which induce the maturation of APCs. We developed artificial antigens by appending the peptide motifs, which have been reported to have agonistic activity for Toll-like receptor 4 (TLR4), to create "adjuvant-free" antigens. The created antigens with triple TLR4 agonistic motifs in their C termini have an activated nuclear factor kappa B signaling pathways through TLR4. These proteins also induced the production of the inflammatory cytokine tumor necrosis factor α and the expression of the co-stimulatory molecule CD40 in APCs, supporting the maturation of APCs *in vitro*.

Association of soluble PD-L1 in blood and PD-L1 expressed on peripheral immune cells in patients with advanced pancreatic cancer

We have found that plasma levels of soluble (s) PD-L1 in patients with advanced pancreatic cancer are significantly higher than those in healthy subjects. We have also found that PD-L1 expression in peripheral immune cells is higher in patients with cancer than in healthy subjects and that PD-L1 expression is highest in CD4⁺ T cells. The sPD-L1 was detected with enzyme-linked immunosorbent assay (ELISA) in the culture supernatants of CD4⁺ T cells from patients with pancreatic cancer, suggesting that sPD-L1 in blood is derived from CD4⁺ T cells. The immune-suppressive activity of sPD-L1 is now being investigated.

sPD-L1 may become a prognostic marker for human lung cancer

Plasma levels of sPD-L1 in 96 patients with lung cancer, including 73 with adenocarcinomas, 7 with squamous cell cancer, 1 with large cell cancer, and 15 with small-cell cancer, were examined with ELISA. Our results showed that plasma levels of sPD-L1 were higher in patients with lung cancer than in healthy subjects and that overall survival and progression-free survival of patients with high plasma levels of sPD-L1 were significantly shorter than those of patients with low plasma levels of sPD-L1. These results suggest that the plasma level of sPD-L1 may become a prognostic marker for patients with lung cancer.

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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

Increased itching sensation depends on an increase in dorsal root ganglia interleukin 31 receptor A expression in mice with atopic-like dermatitis

Although itching and scratching are important factors in the development of atopic dermatitis, the mechanisms that underlie these phenomena are poorly understood. Cohousing with skin-lesioned NC/Nga mice, an animal model of atopic dermatitis, gradually increased itch-associated scratching behavior (long-lasting scratching, LLS) counts in several strains of mice. On the other hand, a repeated dose of interleukin (IL) 31 gradually increased LLS counts by increasing the expression of IL-31 receptor A (IL-31RA) from the dorsal root ganglia (DRG) of mice. We investigated the relationship between the LLS counts and the expression of IL-31 and IL-31RA messenger (m) RNA in the skin and DRG of mice. The LLS counts were significantly increased in NC/Nga and BALB/c mice after 3, 7, and 14 days cohoused with skin-lesioned NC/Nga mice, in a durationdependent manner. Cohousing with skin-lesioned NC/Nga mice significantly increased the expression of mRNA for cutaneous IL-31 and DRG IL-31RA compared with these levels in noncohoused NC/Nga and BALB/c mice, while DRG IL-31 mRNA was not observed. Increased LLS counts were closely correlated with increased DRG IL-31RA mRNA expression, but not with cutaneous IL-31 mRNA expression, in NC/Nga and BALB/c mice.

Moreover, these phenomena were also observed in W/Wv and Scid mice after 2 weeks of cohousing with skin-lesioned NC/Nga mice. The expression of DRG IL-31RA was significantly higher in cohoused NC/Nga mice than in noncohoused NC/Nga mice. A single dose of IL-31 significantly increased LLS counts more clearly in cohoused NS/Nga mice than in noncohoused NC/Nga mice. These results suggest that IL-31–induced LLS is enhanced by DRG IL-31RA expression in mice and that cohousing-induced itching is regulated by DRG IL-31RA expression, as in the case of itching induced by repeated administration of IL-31.

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 the present study was to investigate whether oral immunotherapy with a 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 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 or 20 g of transgenic rice or normal rice.

No major adverse effects were observed in either group during treatment. Allergen-specific T cells 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 mono-nuclear cells after the subjects had eaten transgenic rice. Taken together, oral immuno-therapy and a small dose of the transgenic rice are expected to be an effective treatment for cedar pollinosis.

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 cytotoxic T lymphocytes (CTLs) 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 CTLs by cross-priming were observed. Then antitumor activities were measured with vaccinations with this adjuvant and an 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 kinds of tumor by vaccination.

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Research Center for Medical Sciences Division of Medical Engineering

Masayuki Yokoyama, Professor and Director

Kouichi Shiraishi, Assistant Professor

General Summary

The Medical Engineering Laboratory provides new and essential techniques for developments of medical treatment. We have developed a new concept for an acute ischemic stroke treatment. For this project we have collaborated closely with clinical departments and basic science laboratories, both in our university and hospitals and others. There is a key technology in our laboratory: polymeric micelle carrier systems. Polymeric micelle carrier systems are nano-sized drug carriers for both therapeutic drugs and diagnostic drugs. We have applied a polymeric micelle system to a magnetic resonance imaging (MRI) contrast agent for diagnosis in acute ischemic stroke. Owing to a risk of hemorrhage related to recombinant tissue plasminogen activator (rt-PA) in acute ischemic stroke, we have examined the permeability of the blood-brain barrier (BBB) to increase the safety of treatment with rt-PA. We found that the polymeric micelle MRI contrast agent exhibits an area with a hyperpermeable BBB in a rat transient middle artery occlusion model, and this polymeric micelle MRI contrast agent-based diagnostic system can improve the efficiency of rt-PA therapy. We will perform further experiments to show a relationship between the diagnosis with polymeric micelle MRI contrast agent's and the efficiency of rt-PA therapy. The polymeric micelle carrier systems have great potential for therapy when combined with the diagnosis for cancer and acute ischemic stroke.

Research Activities

Polymeric micelle drug carrier systems in ischemic stroke

Self-assembly block copolymers have been actively studied for drug targeting. Professor Yokoyama, the director of this laboratory, is an international pioneer in the development of self-assemblies of synthetic block copolymers, polymeric micelles, for anticancer drug targeting systems. We are attempting to develop the next generation of novel technology based on the polymeric micelle carrier systems.

We have shown that the polymeric micelle MRI contrast agent possesses the abilities to accumulate in target tissues where the tissues exhibit hyperpermeable vasculature and to provide high-contrast images in the tissues. We have been studying a novel concept of the polymeric micelle MRI contrast agent for the diagnosis of acute ischemic stroke. In cases of acute ischemic stroke, rt-PA is the only drug for treatment. However, rt-PA is known to increase the risk of hemorrhage. For safe use of rt-PA, a novel diagnostic concept is highly desired. In a rat model of transient middle cerebral artery occlusion (MCAO) reperfusion, it is well established that BBB exhibits hyperpermeability of macromolecules, such as serum albumin and other proteins. We focused on the hyperpermeable BBB area where macromolecules exhibit extravasation. We applied the polymeric micelle MRI

contrast agent to detect the hyperpermeable BBB area. In a 3-hour MCAO-reperfusion model, the polymeric micelle MRI contrast agent showed high-contrast foci within a part of the core of the ischemic hemisphere. The high-contrast foci did not completely overlap with the area of edema where both diffusion-weighted imaging and T_2 -weighted imaging were provided. A conventional low-molecular-weight MRI contrast agent did not show such images as the polymeric micelle MRI contrast agent did. The results indicate that the polymeric micelle MRI contrast agent exhibits hyperpermeable BBB foci in ischemic hemisphere, and such foci have never been obtained clearly. Therefore, the polymeric micelle MRI contrast agent has great potential to assess a condition of acute ischemic stroke, namely the condition of hemorrhage risk after rt-PA treatment. We studied the time dependence of the permeability of BBB after MCAO-reperfusion by using the polymeric micelle MRI contrast agent and have found that BBB exhibits hyperpermeability in a time-dependent manner. Furthermore, we must optimize the MRI contrast agent system to assess the risk of BBB. We have started to synthesize new polymer-based MRI contrast agents to optimize the hyperpermeable BBB. We proved that the polymeric micelle carrier system will be useful for the diagnosis of acute ischemic stroke, and a the diagnostic and therapeutic approaches will be combined. This is our new and valuable challenge for next year.

Polymeric micelle drug carrier systems in immune system

We have been studying poly(ethylene glycol) (PEG)-related immune responses. The phenomenon exhibits a specific immune response for PEG, which is used for hydrophilic coverage of typical drug carriers. Previous reports have shown that PEG-liposomes induce a PEG-specific IgM antibody (anti-PEG IgM). We have confirmed that anti-PEG IgM is generated when PEG-poly(β -benzyl l-aspartate) (PBLA) block copolymer micelles (PEG-PBLA micelles) are intravenously injected. However, we have obtained 2 interesting results regarding the behavior of anti-PEG IgM. First, no results prove anti-PEG IgM binding to the PEG main chain, whereas the antibody is called anti-PEG IgM. To examine anti-PEG IgM binding behavior, we synthesized new PEG-triblock copolymers. We found that anti-PEG IgM exhibits bindings to PEG-coated plates where PEG chains were directly attached to the plates. However, anti-PEG IgM could not strongly bind to PEG-coated plates where PEG chains were indirectly coated. The results indicate that anti-PEG IgM exhibits specificity for a PEG chain; however, anti-PEG IgM does not strongly bind to a PEG chain itself. We found that only anti-PEG IgM can bind to a PEG chain possessing the proximity of hydrophobic blocks. Second, we have found that PEG-PBLA micelles exhibited different behaviors in the presence of anti-PEG IgM, as compared with PEGylated-liposomes' behaviors, although both nanoparticle systems possess PEG. The PEG-PBLA micelles exhibited no change in pharmacokinetics in the presence of anti-PEG IgM, whereas PEG-liposomes exhibited drastic changes in pharmacokinetics.

We attempted to solve this issue with our experiments. The maximum number of generated anti-PEG IgM antibodies was limited. In the presence of the limited number of anti-PEG IgM antibodies, we found that the number of PEG-PBLA micelles was nearly the same as the number of anti-PEG IgM antibodies. In contrast, we found that the number of PEG-liposome particles was one tenth the number of anti-PEG IgM antibodies. Therefore, 10 anti-PEG IgM antibodies bind to each PEG-liposome particle, and anti-PEG IgM-bound PEG-liposomes exhibit a rapid blood clearance. We performed further experiments to show PEG-related immune responses. We have found that the first injection of PEG-PBLA micelle induces anti-PEG IgG and anti-PEG IgM in a dose-dependent manner. We will investigate the PEG-related antibody generation to prove PEG-related immune responses.

Research Center for Medical Sciences Division of Ultrasound Device Development and Application

Norio Nakata, Associate Professor and Director

General Summary

1. Theoretical studies of the mutual relationship of ultrasound and microbubbles Microbubbles are considered an ultrasound contrast agent and enhancer and have come to

play an important role. So that microbubbles are used more effectively and rationally, we have employed the finite-element method and the mesh-free method and have studied various types of simulation of the behavior and the effect of microbubbles in the ultrasonic field.

2. Visualization studies of the *in-vivo* ultrasonic field with magnetic resonance imaging We hope the realization of the monitor of the ultrasonic wave propagation situation to such cheap magnetic resonance imaging equipment human body. We believe that the ultrasonogram has much potential and will become a safer therapeutic medical device.

3. Nanobubbles, molecular imaging research

We have collaborated with the Department of Biochemistry and the Department of Laboratory Medicine (The Jikei University) and the Tokyo University of Science. By using the original nanobubbles, we have studied d-d-s- and cancer treatment and have a plan of in vivo study.

4. Study of breast ultrasound diagnosis using machine learning

The subjects of this study were ultrasonic images of the breast that have resulted in a pathological diagnosis. The images have been input to a computer installed in the research department to develop ultrasonic applications and have been used as teaching images (images with the answer) for machine learning with a computer. By the analysis by entering the image data collected developed machine learning.

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, and the posttraumatic stress disorder. 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 Ca²⁺ 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 of pain-related negative emotion

Using rodent models of chronic pain, such as the diabetic neuropathy model and the formalin-induced inflammatory pain model, we demonstrated robust synaptic potentiation at the excitatory synapses between afferent fibers arising from the lateral parabrachial nucleus and neurons in the central nucleus of the amygdala, a structure playing a principal role in the expression of emotional behaviors. We also demonstrated monosynaptic connection between these nuclei using optogenetics with channel rhodospin-expression systems and measurement of light-evoked postsynaptic responses. Furthermore, we have applied small animal magnetic resonance imaging with an ultrahigh magnetic field scanner to visualize the spontaneous cerebral activities with activity-dependent Mn²⁺ uptake during the establishment of chronic pain. These findings further confirm the notion that the chronification process of pain involves potentiated link between the nociception and emotion in the amygdala.

Synaptic mechanism underlying acquisition and extinction of fear memory

The Pavlovian fear-conditioning paradigm depends on the association between a contiguously applied conditional cue and an unconditional aversive sensation. It has been
unequivocally established that the plasticity in the amygdala network plays the primary role in this associative learning. However, the origin and pathway of the aversive signal in fear conditioning have been only poorly identified. We have demonstrated that optogenetic "artificial" stimulation of the axon terminals in the central amygdala, arising from parabrachial neurons delivered in association with conditional auditory cue, robustly established associative fear/threat learning, even in the absence of aversive sensory inputs. This finding is the first to demonstrate the role of a nonthalamic nociceptive pathway in fear learning.

Publications

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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

Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) have been widely used to treat hyperlipidemia. However, they also have adverse effects on muscle, the liver, kidneys, and other organs. To investigate the incidence of these adverse effects and anti-hyperlipidemic effects, we performed a study according to a case-cohort design in which detailed data were collected from all cases and from a subcohort representing 5% of all subjects. A full-scale study has been completed with a large sample size of 7,000 patients from 68 institutions. A paper describing this study has been published.

The above-mentioned statin study took a long time to complete. 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.

To raise the literacy of clinical trials among researchers we held "Clinical Trial Seminar" 4 times this year. The themes were as follows: "Superiority trial and non-inferiority trial" (October 2015), "Equivalence trial" (Nobember 2015), "Evolution of IRB and clinical trials" (January 2016), and "Clinical trial insurance for compensation to research subjects to meet the demand of Ethical guidelines for medical and health care research involving human subjects" (February 2016).

itoring. 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

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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

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placebo-controlled trial. World Allergy Organ J. 2015; 8: 11.

Research Center for Medical Sciences Division of Clinical Epidemiology

Masato Matsushima, Professor and Director

General Summary

The 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" 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 about the prognosis of patients receiving home medical care in Japan. The EMPOWER-JAPAN study was started as a multicentered prospective cohort study to describe in-home mortality and clarify its predictors. The cohort consists 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 will be up to 4 years. This study is financially supported by the 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 nonspecialists by using the official Japanese version of the assessment form "Assessment of Chronic Illness Care."

Psychological impact of lifestyle-related disease disclosure at general checkup: A prospective cohort study

To clarify the psychological effect on patients by disclosing lifestyle-related disease, we compared the state of anxiety between before and after the explanation of general checkup results by a cohort study in 2 primary-care facilities.

Cohort study of patient's complexity

As the size of the aged population increases, the complexity of patients regarding biomedical and psychosocial issues is considered likely to increase. The aim of the study was to examine the effect of patients' complexity on the length of stay in hospitals by employing the "Patient Centered Assessment Method."

Publications

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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

The Hu proteins (the neuronal Elav-like: nElavl) are the mammalian homologue of *Drosophila* Elav, an RNA-binding protein expressed in the nervous system. In the embryonic brain, Hu family proteins (HuB/C/D) induce neuronal differentiation by binding preferentially to GU-rich sequences with secondary binding to AU-rich sequences in target RNAs. To study the function of HuC in mature neurons, we generated HuC-deficient knockout (HuC KO) mice. At 7 months of age, HuC KO mice exhibited intention tremor, gait abnormality, and ataxia. Before the onset of these symptoms, the axons of Purkinje cells underwent the morphological changes of swelling and retraction at the deep cerebellar nuclei, although the pathological changes were not observed during cerebellar development.

Electron microscopic analyses showed the accumulation of mitochondria, endoplasmic reticulum and cytosolic organelles, such as nuclei and ribosomes, in the swollen Purkinje axons, indicating that a diffusion barrier system between soma and axons might be impaired in HuC KO mice. When the data of electron microscopy and the previous analysis of HuC target RNAs were combined, AnkyrinG was identified as a responsible factor for these pathological phenomena. In neurons, AnkyrinG is distributed to the axon's initial segment and forms the size-dependent diffusion barrier between soma and axons. This system is needed to define the delicate protein distribution in neurons. Our studies have shown that HuC regulates the alternative splicing of AnkyrinG and that the splicing pattern of AnkyrinG is disrupted in HuC KO mice. Intriguingly, the expression level of the embryo-specific variant of AnkyrinG was increased in HuC KO. Furthermore, the embryonic and adult variants of AnkyrinG exhibited differential binding affinity to spec-

trin. This data indicates that HuC maintains the homeostasis of axons by controlling the alternative splicing of AnkyrinG.

The TAR DNA-binding protein of 43 kDa (TDP-43) gene (*TDP-43*) has been identified as a causative gene of both amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), based on the pathological findings as ubiquitin-positive cytoplasmic inclusions containing TDP-43 protein. Point mutations of *TDP-43* have been found in patients with ALS or FTLD. A cause of neuronal cell death in neurodegenerative diseases is abnormal RNA metabolism, although the mechanisms are unclear. Here we generated 2 lines of mutant human *TDP-43* knock-in mice (mTDP-43 KI) and investigated the causal role between the gene mutation and ALS/FTLD phenotypes. Interestingly, considerable differences in the phenotype and pathology between the 2 lines of mTDP-43 KI were observed. Poor weight gain, decrease of motor function, loss of motor neurons, and phenotypes related to ALS were more significant in 1 of the mTDP-43 KI lines. In this line, TDP-43–positive inclusions and cystatin C–positive Bunina bodies appeared in spinal cord motor neurons.

Furthermore, to identify diagnostic biomarkers for ALS, we determined abnormal RNA metabolism in white blood cells of mTDP-43 KI lines. We found that messenger RNAs of both Smn1 and NLR family, apoptosis inhibitory protein 5 (*Naip5*) were candidate targets for further studies.

A primate model of human diseases

The common marmoset (*Callithrix jacchus*), a small New World primate, is becoming increasingly popular in biomedical research, because of its advantage for translation to genetically close human systems. To investigate the use of magnetic resonance imaging diffusion-tensor imaging (DTI) to detect denervation of the nigrostriatal pathway in a marmoset model of Parkinson disease (PD) after treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. The DTI and the tractography showed the loss of fiber structures of the nigrostriatal pathway in the PD model. Our study provides a potential basis for the use of DTI in the clinical diagnosis of PD (Hikishima K et al. *Radiology* 2015). Furthermore, decreases in the volume of the substantia nigra in a marmoset model of PD were detected with voxel-based morphometry and magnetic resonance imaging and confirmed with histologic findings as the degeneration of dopaminergic neurons (Hikishima K et al. *Neuroscience* 2015).

Disease modeling with iPSCs

Pallidopontonigral degeneration, part of frontotemporal dementia with parkinsonism related to chromosome 17, is caused by mutations in the microtubule associated protein tau gene (MAPT) encoding tau protein. We generated iPSCs from a patient with an N279K mutation of MAPT to investigate the underlying disease mechanism. In iPSC-derived neural stem cells, we observed accumulation of endosomes and exosomes and a reduction of lysosomes, which displayed impaired endocytic trafficking. Our experiments demonstrate that alterations of intracellular vesicle trafficking in neural stem cells and neurons likely contribute to neurodegeneration (Wren et al. *Mol Neurodegener* 2015).

Publications

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Research Center for Medical Sciences Core Research Facilities for Basic Science (Division of Molecular Genetics)

Hisashi Yamada, Professor and Director

Yumi Kanegae, Associate 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 adenovirus vectors.

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 demonstrated that bromodomain and extra terminal domain inhibitor 151 (I-BET151) is a promising agent for some myeloid leukemias. The acquisition of resistance to a drug by leukemic cells is a critical hurdle for achieving clinical success. We established I-BET151–resistant U937 cells (U937R) and compared the characterization of these cell lines. Gene expression profiles by means of microarray analysis disclosed no involvement of the ATP-binding cassette genes for the resistance. Because the expression of immune-system–related genes is modulated by I-BET151, we studied the effect of the nuclear factor kappa B pathway on the growth of parental and resistant cells. Both types of cell were treated with I kappa B kinase inhibitor VII (IKKi). The U937R cells treated with low concentrations of IKKi (1 to 4 μ M) were more sensitive than parental cells. Interestingly, 1 μ M of IKKi did not alter the expression of either *MYC* or *BCL2* in U937R cells. These results suggest that the survival of U937R cells is dependent on the nuclear factor kappa B pathway.

Cancer Molecular Biology

To reveal the appearance mechanism of a malignant tumor complicated by a congenital disorder, we used a next-generation sequence to analyze the exhaustive cancer-related gene expression. We reported on a 7-year-old girl with Maffucci syndrome in whom acute myeloid leukemia with a cup-like nuclear invagination had developed. The rate of malignant transformation in Maffucci syndrome is high; however, hematopoietic malig-

nancies rarely arise in this syndrome. In patients with Maffuccie syndrome, somatic mutations of *isocitrate dehydrogenase (IDH)* have been reported. In this patient we found the insertion frameshift at the nucleophosmin gene (*NPM1*) in leukemic cells and hemangioma. These results suggested that the multiple somatic mutations of the *IDH1* and *NPM1* gene in hemangioblasts were related to the development of cup-like acute myeloid leukemia associated with Maffucci syndrome. We also analyzed the medulloblastoma that was complicated with Gorlin syndrome and the Wilm's tumor that was complicated with phacomatosis pigmentokeratotica.

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. We have already established the cell-specific gene expression system using the combination of AdV and site-specific recombinase Cre or FLP. Because AdVs offer a transient expression system, they can also be applied to the differentiation instructions from stem cells to purpose cells. We constructed 16 AdVs for the induction of neuron cells from induced pluripotent stem cells. With this method, the efficacy of motor neuron induction was 7 times greater than with a standard method. Furthermore, because many immature cells remained when induction was being differentiated, we generated the AdVs carrying the drug-resistant gene driven by a neuron-specific promoter. These AdV systems may contribute to the analysis of the cause of the neurologic disease.

We also developed a protocol to cure hepatitis B virus (HBV) infection with an AdV. Current therapeutic treatments for HBV infection most often use nucleotide analogs, such as lamivudine and interferon α . However, these treatments cannot effectively eliminate the covalently closed circular DNA, which present in infected hepatocytes and continue to serve as the template for the pregenomic RNA. Consequently, complete clearance of HBV is difficult. We succeeded in constructing AdVs expressing 3 genomic RNAs targeting the HBV genome. The expressed genomic RNAs together with CRISPR-associated protein 9 using AdVs efficiently direct cleavage and cleavage-mediated mutation occurred in covalently closed circular DNA. These results suggest that an AdV expressing multiplex genomic RNA targeting the HBV genome might be used as an effective tool to treat chronic HBV infection.

Publications

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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 Akito Tsubota, Professor Toshiaki Tachibana, Professor Kouki Fujioka, Assistant Professor

General Summary

The Core Research Facilities were reorganized on April 1, 2014, and its name was changed to Core Research Facilities for Basic Sciences (Division of Molecular Cell Biology). The mission of the facilities is the facilitation of research in the university. Two systems are constituted for the use of the facilities for Basic Sciences (Division of Molecular Cell Biology).

Research Activities

Cell membrane perforation with photosensitizer and a brush-shaped soft-polymer sheet and apoptosis of colon and lung cancer cells by microRNA-203 and Puma expression Transduction of foreign molecules into cells is an important technique for investigating the functions of corresponding molecules or targets or both. Recently, a mass-producible nanoprinting perforator was devised to enable large-scale, high-performance drugs or nucleic acids to be transferred into cells without causing damage. Therefore, we investigated the effects of this perforator on a malignant glioma cell line. Photosensitization transduced fluorescein isothiocyanate-conjugated albumin into cells. The trypan blue inclusion test demonstrated membrane disintegration by the procedure, and scanning electron microscopy disclosed perforation of the cell membrane. A local oxidation reaction during nanoprinting caused reversible membrane perforation; therefore, the specific printing system might be convenient for the transduction of foreign molecules into malignant glioma cells.

The relationship between microRNA-203 (miR-203) and the p53 upregulated modulator of apoptosis (Puma) was investigated in colon and lung-cancer cell lines. Although p53 downregulation decreased both miR-203 and Puma expression, miR-203 overexpression increased Puma expression. These findings suggest that activated p53 increases both miR-203 and Puma expression remains elevated in cells with miR-203 overexpression in the presence of p53 downregulation. Our data suggest that p53 increases Puma expression directly and may also do so through miR-203. This functional study revealed that miR-203 overexpression induces apoptosis and inhibits cell invasiveness.

Chimeric model mice of hepatitis infection with human hepatocytes, intrahepatic cellular localization of ATP7B, gene mutation in the treatment of chronic hepatitis C virus infection, comprehensive gene expression profiling analysis of microRNA/messenger RNA, and gene delivery and immunomodulatory effects of plasmid DNA associated with branched amphiphilic peptide capsules

We have established the human hepatocyte chimeric mouse as an animal model for investigating hepatitis B or C virus infection and are aggressively researching the efficacy of novel antiviral agents, the infection mechanism, and the ultrastructural alteration of intrahepatocellular organelles after viral eradication.

In collaboration with the University of Barcelona (Spain), we are investigating the protein ATPase copper transporting beta (ATP7B), which balances the copper level by excreting excess copper into bile and plasma, because the exact localization of ATP7B in the hepatocyte remains to be determined.

We are investigating the association of single nucleotide polymorphisms of the genes with the serum drug concentration, treatment response, and liver damage induced by directly acting antiviral agents in the treatment of hepatitis C virus infection. Resistance-associated variants are also being investigated in detail.

We have found the novel interaction between microRNA and messenger RNA in the replication and life cycle of hepatitis B virus and investigated the association of the serum microRNA expression level with treatment outcomes and prognosis in patients with hepatocellular carcinoma who were treated with transcatheter arterial chemoembolisation and radiofrequency ablation. In addition we have reported on a new class of branched amphiphilic peptides associated with double-stranded DNA and promoted *in vitro* transfection of eukaryotic cells, yielding high transfection rates and minimal cytotoxicity and representing a new and promising nonviral DNA/gene delivery approach for DNA vaccines.

Matrix-remodeling response of human periodontal tissue cells toward fibrosis upon nicotine exposure

Fibrosis is frequently observed in the gingiva of smokers. However, the mechanisms by which smoking results in pathological changes in periodontal tissue and lead to fibrosis are not entirely clear. Our former report showed that type I collagen synthesis is promoted by nicotine via CCN family protein 2 in human periodontal tissue cells. Here, we evaluated other aspects of nicotine function from the viewpoint of extracellular matrix remodeling. Human gingival fibroblasts (n = 4) and periodontal ligament cells (n = 3) were isolated. The cells were treated with various concentrations of nicotine for 12 to 48 hours. Modulators of matrix remodeling were measured with enzyme-linked immunosorbent assays. Cell migration and morphology were also evaluated. After treatment with 1 µg/ml nicotine, significant increases (p < 0.05) were observed of tissue inhibitor of metalloproteinase 1 and transforming growth factor β 1 production in both cell lysates and supernatants and of matrix metalloproteinase 1 production in cell lysates. Cell migration was significantly inhibited (p < 0.005) by nicotine in a time-dependent manner. Electron microscopic analysis revealed vacuoles in nicotine-treated cells. These results indicate that nicotine impairs fibroblast motility, induces cellular degenerative changes, and alters the extracellular matrix remodeling systems of periodontal cells. Induction of matrix remodeling molecules, combined with type I collagen accumulation, may account for the molecular mechanism of nicotine-induced periodontal fibrosis.

Control of insulin secretion by urocortin III under hyperglycemic condition

Insulin secretion from pancreatic β cells is reported to be disturbed under hyperglycemic conditions. A recent study has found that release of urocortin III, a specific antagonist of corticotropin-releasing factor receptor type 2, both stimulates insulin release and is stimulated by elevation of extracellular glucose. Therefore, the effect of urocortin III at higher glucose levels on insulin release was investigated with the pancreatic β cells of MIN6 mice. The addition of urocortin III (10⁻⁷ M) resulted in a gradual increase of insulin release but in a decrease in a culture medium with 90 mM glucose. We will further investigate the mechanism of urocortin III–induced insulin release under such hyperglycemic conditions.

Development of an in vitro brain model for nano-brain toxicology assay

Recent technical innovations have allowed various nanomaterials to be mass-produced. Although nanomaterials are used for daily-use materials, such as foods and cosmetics, because of improved quality, nanomaterials are still being investigated for their safety. Recently, we have investigated the effect of nanoparticles on several brain cells. This year, we investigated the penetration mechanism of nanoparticles into the blood brain barrier using endothelial cells of capillary vessel. Our data showed that the cell index (electrical resistance value) of endothelial cells was decreased with the addition of nanoparticles, although the observation images of the cells were apparently unaffected. This result suggests that the barrier function of endothelial cells is affected by the nanoparticles and allows the particles to penetrate. Because the cell index seems to have higher sensitivity than does the observation of cell images, we will use the index to screen for the penetration of particles.

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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 2015, 657 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, the bloodstream form is taken up and differentiates to the procyclic form in the midgut. Subsequently, procyclic forms migrates to the tsetse salivary gland or proboscis where they differentiate to epimastigote forms. Then, at the end of tsetse infection, the epimastigote form differentiates into the animal infective metacyclic form, which differentiates to the bloodstream form after being injected into vertebrate hosts. Although the cell differentiations between each lifecycle stage are considered promising targets for developing novel control measures against the disease, their molecular mechanisms have not been elucidated. Previously, protein tyrosine phosphatase 1 (PTP1) has been reported to be involved in the cell differentiation regulations of trypanosomatid parasites Trypanosoma brucei (bloodstream form to procyclic form) and Trypanosoma cruzi (epimastigote form to metacyclic form). Currently, we focus on the PTP1 of Trypanosoma congolense, the cause of animal African trypanosomiasis. Because all lifecycle-stage developments of T. congolense are reproducible in vitro, with the use of the parasite, more detailed biological functions of the protein could be studied. So far, the T. congolense PTP1 has been cloned, and its expression pattern and phosphatase activity were investigated with biochemical analyses. We are now studying the biological functions of the T. congolense PTP1, especially its roles in the regulation of cell differentiation.

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 the *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 *Plasmodium* infection causes drastic alteration of plasma aminograms and that the treatment of mice with food in which the amino acid composition has been modified significantly inhibits parasitemia. Furthermore, a combination study with artificial food and artesunate, which is the first-line drug against malaria, indicated that this food has a synergistic effect with antimalarial agents. Currently, in an *in vivo* murine model, we are studying the presence or absence of the association between plasma aminograms and cerebral malaria, which is one of the most severe clinical manifestations of malaria.

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Research Center for Medical Sciences Radioisotope Research Facilities

Kunihiko Fukuda, Professor and Director Haruka Minowa, Assistant Professor Tadashi Asakura, 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 2015, 36 researchers from 10 departments and 16 students of 2 curriculums used the laboratory of this facility. Major nuclides used for experiments were ³²P, ⁵¹Cr, ¹²⁵I, ¹⁴C, and ³H.

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 radio-active 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

E-cadherin suppression in epoxomicin-resistant cells may be regulated by expression of zinc finger E-box-binding homeobox 1

The Ish/EXM strain, comprised of endometrial carcinoma Ishikawa cells resistant to epoxomicin, a proteasome-specific inhibitor, was established, and several features were examined to overcome the resistance to treatment.

The 50% growth inhibition concentration for epoxomicin against Ishikawa cells and Ish/ EXM cells was 20 and 400 nM, respectively. The Ish/EXM cells have also acquired resistance to the proteasome inhibitors MG132, PSI, and PS-341.

Acquiring resistance to epoxomicin led to the disappearance of both E-cadherin 1 gene (*CDH1*) messenger (m) RNA and E-cadherin protein in Ish/EXM cells and to the induction of epithelial-mesenchymal transition (EMT) in Ish/EXM cells. Because E-cadherin protein expression is regulated by a transcriptional suppression factor, we used the reverse transcriptase–polymerase chain reaction to measure mRNA expression of several factors related to E-cadherin suppression, specifically Snail, Slug, zinc finger E-box-binding

homeobox 1 (ZEB1), ZEB2, E12/E47, and Twist, in Ish/EXM cells. Among these suppressors concerning epoxomicin induction, expression of ZEB1 and ZEB2 was especially enhanced in Ish/EXM cells, and expression of the ZEB1 protein was markedly increased. On the other hand, because E-cadherin was suppressed in Ish/EXM cells, we expected EMT to be induced in these cells. Therefore, we measured the migration of both Ishikawa and Ish/EXM cells. The invasive capacity of Ish/EXM cells increased to 5.7 times that of Ishikawa cells.

To identify the primary transcriptional suppressor for E-cadherin suppression, the factors Snail, Slug, ZEB1, ZEB2, and Twist were knocked down in Ish/EXM cells. Treatment of Ish/EXM cells with ZEB1 small– interfering (si) RNA but not with ZEB2, Snail, Slug, or Twist siRNA restored the expression of both E-cadherin mRNA and E-cadherin protein. Of note, treatment of Ish/EXM cells with ZEB2 siRNA partially restored expression of E-cadherin mRNA.

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. The X-ray–irradiated cells show a longer tail than did the control nonirradiated cells. However, because the observed results were not quantified automatically, the experimental operation needs to be improved.

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 with a liquid scintillation counter. With this method, the chemical separation of 24 hours could be evaluated and compared with 2 weeks with a conventional technique. The detection limit in this procedure from 1 liter 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 concentration of spring waters designated as the 57 best waters by the Tokyo Metropolitan Government Bureau of Environment. The results of this study were presented at the 68th annual meeting of the Japanese Society of Hot Spring Sciences (September 2015, Yamagata, Japan).

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 the maintenance of the GMP standard in the facility.

Research Activities

Immune cell therapy, using dendritic cell vaccines against glioblastoma multiforme and pancreatic cancer, and regenerative therapy, using cell sheets from nasal mucosa for tympanoplasty after pearl tumor surgery in the ear, have been successfully performed at this GMP cell-production center. Because a law requiring that the safety of regenerative therapy be assured was enforced on November 25, 2014, our activities of cell therapy or regenerative medicine will be regulated. A working group will establish a committee for third-class regenerative medicine. This GMP cell-processing center must be certificated by the Ministry of Health, Labour and Welfare as a cell-culture facility for regenerative medicine. The devices in this center will be reevaluated by the inspection of the Pharmaceuticals and Medical Devices Agency.

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 for 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 (MRI).

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.

This year, we have started evaluating the deformation of a skeletal muscle model using MRI in the development of a 4D human body model with deformable soft tissue (skin, abdominal organs, skeletal muscle, and vascular systems) during whole body motion. In the evaluation, we have developed a dynamic MRI measurement method that can measure in the same situation as when the body walks, with the same load applied. We are comparing the deformation of the real skeletal muscle of the lower limb during walking motions and the skeletal muscle model's deformation by measuring the deformation of the real skeletal muscle in 4D. In addition, by using various X-ray CT data we have previously measured, we are developing a system that visualizes the future growth of a child. This research is being performed by departments in our university in collaboration with Osaka University, Kyushu University, and Mayo Clinic (Rochester, MN, USA).

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. 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, we are also continuing our development of the multiple viewpoint camera system for endoscopic and robot surgeries.

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, to advance the integration of the simulator with the surgical navigation system we are developing, we started the development of a 4D imaging display system that enables the surgeon to instinctively grasp the patient's biological structure and its dynamics.

Development of an image-guided surgery system

We are developing a system that can display blood vessels and tumors at the back of a surgical field in the form of 3D geometric models in multiple layers on a 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.

This year, we developed a new display system. A surgeon can now view real-time navigation information from a tablet personal computer. We applied the system to clinical use.

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 performed 3D analyses of the position, depth, and angle of the attempted-murder victim's injuries using the victim's X-ray CT data set.

This research was performed in collaboration with our university's Department of Forensic Medicine, the Tokyo District Prosecutor's Office, and the Metropolitan Police Department.

Publications

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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 2015 we started an educational laboratory course program with the assignment of third-year students from the School of Medicine. We also fulfilled research support duties for registered researchers from Kashiwa University Hospital (Departments of Gastroenterology; Laboratory Medicine; Neurosurgery; Diabetes, Metabolism and Endocrinology; and Obstetrics and Gynecology) so that physician-researchers could work freely. Their research work has progressed efficiently.

Research Activities

Mechanism of islet injury and beta cell regeneration in diabetes mellitus

Although most cells in the pancreatic islets of Langerhans (pancreatic islets) are derived from endodermal endocrine cells, the islet structure also includes peripheral nerve fibers that are non-endocrine cells, capillaries, and ectoderm oriented, such as neural crestderived peri-islet Schwann cells. The Schwann cell might have the same functions as allogeneic astrocytes and Schwann cells in other tissues of the nervous system. These functions might include the supplementation of nutrients to the blood vessels and endocrine cells and shielding cells from exogenous stress, but the functions remain unclear. Helpful research might be to elucidate the structure-function relationship of the islet compartment structure and to understand the origins of pancreatic islet failure in diabetes. Under this concept of "self-organization of the islet" in 2014, we started a study of "beta cell protection from metabolic stress." Experiments showed that, in the co-culture conditions of MIN6, a murine beta cell line, and IMS32, a murine Schwann cell line, glucosestimulated insulin secretion or insulin secretory capacity was significantly higher than in MIN6 of a single culture system. These phenomena were considered protective effects from Schwann cells. We are planning to identify the mechanism of the increased capacity of glucose-stimulated insulin secretion.

Study of glucose and lipid metabolism through novel technology of biological gas measurements

Continuing from the previous fiscal year, we searched for a method of detection with skin-derived gas-by-gas chromatography.

Study of the change of body components during treatment of diabetes mellitus by sodiumdependent glucose co-transporter inhibitor

In the treatment of type 2 diabetes with dietary restrictions and medication, changes of

body composition associated, in particular, with the possibility of muscle loss and body fat increase, have become a problem. Treatment with sodium-dependent glucose co-transporter (SGLT2) inhibitor, a novel oral antidiabetic agent, is expected to cause body weight loss due to fat reduction, but details of the 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 started a multicenter, open-label follow-up study of an SGLT2 inhibitor in Japanese patients with type 2 diabetes. So far 11 medical facilities are involved in this prospective study. In the preliminary analysis, body fat mass was found with dual-energy X-ray absorptiometry to have continued to decrease efficiently for as long as 24 weeks. Skeletal muscle mass decreased slightly during the initial 12 weeks yet showed no further decrease after 12 weeks. We plan to extend the study to evaluate several variables after 1 year.

Publications

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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. The JCNP was created to advance clinical and biomedical research 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; FY2013-2018).

Pain is one of the most serious concerns in medicine. Besides being a beneficial alarm for on-going harmful events, such as the injury and inflammation, pain is not only a simple sensation but it is also inevitably "painful," causes suffering, and is accompanied by strong negative emotions. Such emotional aspects of pain also cause patients to remember the potentially harmful situations. However, such an emotional aspect of pain also decreases quality of life, prevents concentration and rest, and leads to various mental disorders, including depression and anxiety. These changes often lead to various psychosomatic complications. Thus, identifying the neural 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. 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 have (>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/pharmaco-genetics. 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 causes 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 headquarters of the JCNP are located. In addition, advanced experimental systems for pain evaluation and brain activity measurement are in the Department of Neuroscience and are now frequently used by many researchers belonging to the JCNP.

Centers of Advanced Medicine Center for Medical Entomology

Hirotaka Kanuka, Professor and Director Tatsuya Sakurai, Assistant Professor Kenji Ishiwata, Associate 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, because it is one of the most effective means of dealing with the problem while waiting for a vaccine or other effective dengue-control strategy. In this center, based on collaboration with institutions in endemic countries, such as Burkina Faso, Nigeria, and Taiwan, entomological studies promoting multilateral approaches have been performed to gather knowledge of diagnosis, ethology, immunity, and epidemiology of vector species for effective vector control.

Research Activities

Evidence of vertical transmission of severe fever with thrombocytopenia syndrome virus in field-collected ticks

Tick-borne diseases represent major public health issues worldwide. Severe fever with thrombocytopenia syndrome virus (SFTSV) is a newly identified *Phlebovirus* in the *Bun*vaviridae family causing acute hemorrhagic fever in East Asia, China, Korea, and Japan. Cases of SFTSV have been detected and isolated from diverse species of tick in the endemic areas. We collected ticks from 15 localities (over an area of 10 km²) on Kyushu Island, Japan, in April and October 2013. In addition, the localities are known to have an abundance of ticks and a recent history of human cases of Japanese spotted fever. A total of 1,168 questing ticks were collected with flagging vegetation (using a white flannel cloth of 170×70 cm). The SFTSV-specific RNA was detected with the reverse-transcription polymerase chain reaction in complementary DNA generated from the RNA of individual ticks. The SFTSV-specific RNA was detected in 4 species: Haemaphysalis formosensis, Haemaphysalis longicornis, Haemaphysalis flava, and Haemaphysalis hystricis. The SFTSV RNA was not detected with the reverse-transcription polymerase chain reaction in any field-collected Amblyomma testudinarium tick. Most of the ticks collected in April were nymphs (n = 794, SFTSV-positivity = 9.8%), 73 (8.2%) were adults, and only 10 (0%) were larvae. In contrast, in October we collected 8 (0%) nymphs, 38 (0%) adults, and 245 (7.3%) larvae. Interestingly, SFTSV-specific RNA was also detected in larvae. Larvae may become infected with the SFTSV via vertical transmission.

Genetic dissection of intermediate host and tapeworm interaction

Dwarf tapeworm, *Hymenolepis nana*, which belongs to the order Cyclophyllidea, is the most common cestode of humans. Its intermediate host is arthropods, in particular, beetles. Once the intermediate host ingests tapeworm eggs, oncospheres immediately hatch and pass through the insect's gut wall. Cysticercoids develop within the hemocoel, where they survive without loss of infectivity until the intermediate host is ingested by a definitive host. To dissect the interaction between a tapeworm and an intermediate host, we employed a reverse genetic approach with the red flour beetle, Tribolium castaneum, in which a robust systemic RNA interference (RNAi) response is observed, as a model system to explore host responses to tapeworm infection. Adult knock-down phenotypes in T. castaneum were induced by injection of double-stranded RNA into late instar larvae. We performed RNAi screening targeting several gene transcripts of Toll and immune deficiency pathways, which are 2 major signaling pathways of the humoral immune response in insects. Reduction of Toll pathway function, which was induced by RNAi-mediated silencing of MyD88, Dif1, and Dif2, in addition to Janus kinase/signal transducer and activator of transcription and c-Jun N-terminal kinase components, increased the burden of cysticercoids. On the other hand, RNAi-mediated knock-down of the immune deficiency pathway components dredd and imd had no significant difference on cysticercoid load. Our findings suggest a pivotal role of specific pathways, such as Toll signaling, in regulating the resistance to tapeworm infection.

Publications

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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

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 distinguishing 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 Physical 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 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.

Centers of Advanced Medicine Stable Isotope Medical Application Center

Tomokazu Matsuura, Professor and Director Takashi Okano, Professor Koji Nakada, Associate Professor Madoka Nakagomi, Assistant Professor Takeo Iwamoto, Professor Koji Takada, Professor Youichiro Kusakari, Assistant Professor

General Summary

The metabolic study in a living body using stable isotope labeling compounds is useful for medical analysis. Many tests of biological functional which are safe, rapid, and highly sensitive will be developed for clinical use by using stable isotope labeling compounds. We will put stable isotope tests of clinical function to practical use to analyze energy metabolism, nutritional absorption, external secretion from the pancreas, and pharmacokinetics. The systematic translational research leading to clinical application from fundamental studies, including the development of new stable isotope labeling compounds, cells, and animal studies, is necessary to accelerate the practical use of stable isotope biology for functional evaluation. For this purpose, we have performed a project entitled "Building for the Stable Isotope Applicable Medicine" which is supported by the High Technology Research Center Project for Private University from the Ministry of Education, Culture, Sports, Science and Technology of Japan (2011 to 2015). We have also established the Stable Isotope Medicine Application Center in The Jikei University. This center will support the chemosynthesis of stable isotope compounds, basic experiments using cells and animals, and clinical application studies.

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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) the importance of extracellular RNA in bacterial biofilms, 2) redundancy and complexity in biofilms, 3) identification of anti-biofilm compound (ABC)–JK2, a small molecular inhibitor of staphylococcal biofilm formation, 4) visualization of biofilms in solution by atmospheric scanning electron microscopy (SEM), 5) biofilm formation mechanisms triggered by excreted molecular chaperones, and 6) genotypic and biofilm profiles of *Propionibacterium acnes* isolated from pacemakers without clinical signs of infection.

Research Activities

Importance of extracellular RNA in bacterial biofilms

We recently explored the presence of extracellular RNA in *Staphylococcus aureus* biofilms. In this study, we analyzed the roles of extracellular RNA in biofilm development. The molecular size of the extracellular RNA was estimated with urea–polyacrylamide gel electrophoresis to be 20 to 200 nucleotides. We observed localization of extracellular RNA in the 3-dimensional structure of biofilms with confocal laser scanning microscopy. In addition, RNase A inhibited biofilm formation and dispersed preformed biofilms, representing the importance of extracellular RNA in the structural integrity of biofilms. The extracellular RNA was also identified in a biofilm formed by *Pseudomonas aeruginosa*, indicating that this phenomenon is not limited to staphylococcal biofilms. Our findings provide evidence of a novel function for RNA that has important implications for understanding biofilm physiology.
Redundancy and complexity in biofilms

We detected the clinically isolated strain of *S. aureus* that has capacity to form robust proteinaceous biofilms. This strain has a large amount of extracellular adherence protein (Eap), a secreted protein specific to *S. aureus*, in the biofilm matrix. Although we predicted that Eap contributed to biofilm formation, single knock-out of the Eap gene (*eap*) had little effect on the biofilm biomass. We then deleted other genes involved in biofilm formation in the Δeap strain. We found that deletion of a few genes significantly reduced biofilm formation. One of the genes was the sortase A gene (*srtA*), which encodes sortase A, a membrane protein responsible for the binding of some proteins harboring the leucine-proline-any-threonine-glycine motif to the cell wall. Hence, we predict that one or more of these cell wall–anchored proteins has function similar to that of Eap. Elucidation of the role of these proteins will lead to the development of a specific treatment for infection with *S. aureus*.

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 screening hit compounds, named ABC-JK2, inhibited biofilm formation of several strains of *S. aureus*, including methicillin-resistant *S. aureus*, and *S. epidermidis* at a half-maximal inhibitory concentration range of 12.0 to 22.5 μ M. Metabolomic analysis showed that ABC-JK2 decreased intracellular levels of glycolytic metabolites. The microarray/quantitative real-time polymerase chain reaction revealed up-regulation of genes related to peptidoglycan biosynthesis and hydrolases. In addition, transmission electron microscopy showed that the thickness of bacterial cell walls and the number of abnormal septa are increased in the presence of ABC-JK2. These results suggest that ABC-JK2 inhibits staphylococcal biofilm formation by affecting glycolysis and cell-wall synthesis.

Visualization of biofilms in solution by atmospheric SEM

We visualized biofilms immersed in aqueous solution, including biofilms formed by the Gram-positive coccus *S. aureus* and the Gram-negative bacillus *Escherichia coli* by recently developed atmospheric SEM. Because atmospheric SEM allows a biofilm cultured on electron-transparent film windows to be observed from below with an inverted SEM, biofilm formation was able to be studied near the substrate and the extracellular matrix at high resolution. Membrane vesicles, delicate spiral flagella, straight curli fibrils, and filamentous extracellular DNA networks were visualized with atmospheric SEM and effective labelling methods, such as labelling with positively charged Nanogold, heavy metals, and immuno-gold. Collectively, our results suggest that atmospheric SEM is a broadly applicable approach for microbial research and diagnostic purposes.

Biofilm formation mechanisms triggered by excreted molecular chaperones

Previously, we identified excreted cytoplasmic molecular chaperones DnaK and ClpB that exist in the extracellular matrix of *S. aureus* biofilms and promote biofilm formation. Mutational analysis for DnaK demonstrated that the N-terminal nucleotide binding

domain alone stimulated biofilm formation of *S. aureus*. In addition, neither mutations into amino acid residues involved in ATPase activity nor substrate-binding affected biofilm-promotion ability. These results suggest that DnaK acts independently as a biofilm-promoter on functions as a classical molecular chaperone involved in the control of intracellular protein homeostasis.

Genotypic and biofilm profiles of P. acnes isolated from pacemakers without clinical signs of infection

The colonization of bacteria on the surfaces of cardiac pacemakers explanted in patients without clinical evidence of infection was consecutively analyzed. The *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 differed among strains regardless of the sequence types; however, extracellular DNA was suggested to be a factor commonly involved in the biofilm formation of diverse *P. acnes* strains. High-resolution observation of nanostructures in the biofilms with transmission electron microscopy and atmospheric SEM visualized the leakage of cytoplasmic components along with cell lysis and fiber structures connecting cells in biofilm.

Publications

Chiba A, Sugimoto S, Sato F, Hori S, Mizunoe Y. A refined technique for extraction of extracellular matrices from bacterial biofilms and its applicability. *Microb Biotechnol.* 2015; **8**: 392-403. *Koyama R, Okuda K, Matsushita K, Beppu M,*

Mizunoe Y. Antimicrobial and antibiofilm effects of ozonated water for prevention and treatment of bone and joint infections. *Journal of St. Marianna University.* 2015; **6:** 1-7.

Sugimoto S, Arita-Morioka K, Mizunoe Y, Yamanaka K, Ogura T. Thioflavin T as a fluorescence probe for monitoring RNA metabolism at molecular and cellular levels. *Nucleic Acids Res.* 2015; **43:** e92.

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 31 protocols of consultation from April 2015 through March 2016. Consultations were as follows: 15 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), 1 protocol for the application of intellectual property, 3 protocols for the response to reviewers after the submission of articles (including additional analyses), 2 protocols for development strategy, and 10 protocols for conducting statistical analysis. Consultations were requested by the Departments of Endoscopy, Anesthesiology, Neurosurgery, Orthopaedic Surgery, Radiology, Psychiatry, Dentistry, Pediatrics, Forensic Medicine, Otorhinolaryngology, Surgery (Division of Digestive Surgery), and Internal Medicine (Divisions of Diabetes, Metabolism and Endocrinology; Clinical Oncology/Hematology; Gastroenterology and Hepatology; and Cardiology), the Laboratory Animal Facilities, 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" 4 times to improve literacy about clinical trials among researchers. The themes were "Superiority trial and non-inferiority trial" "Equivalence trial," "Evolution of IRB and clinical trials," and "Clinical trial insurance for compensation to research subjects to meet the demand of Ethical guidelines for medical and healthcare research involving human subjects." 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 consider applying a method of analyzing data on competing risks in such a situation. We define an event of improvement on the measurement objectively and treat missing as a competing risk. We explored design in superiority trials and explored statistical methods in noninferiority 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 and different interindividual variations due to different scales of the measures 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

Kawamori R (Juntendo Univ Grad Sch Med), Kaku K(Kawasaki Med Sch), Hanafusa T (Osaka Med Coll), Ioriya K (Sumitomo Dainippon Pharma Co., Ltd.), Kageyama S, Hotta N (Chubu-Rosai Hosp). Clinical study of repaglinide efficacy and safety in type 2 diabetes mellitus patients with blood glucose levels inadequately controlled by sitagliptin. J Diabetes Investig. 2016; **7:** 253-9.

Kiyomi F¹, Nishikawa M, Yoshida Y¹, Noda K¹ (*[†]Fukuoka Univ*). Comparison of intra-individual coefficients of variation on the paired sampling data when inter-individual variations are different between measures. *BMC Resh Notes.* 2016; **9:** 115.

Premedical Course

Biology

Koji Takada, Professor

Rie Hiratsuka, Associate Professor

General Summary

Our research themes are the mechanisms of cellular protein aggregation associated with heavy metal toxicity and the mechanism by which the generative cell of angiosperm is taken into a pollen tube cell.

Research Activities

Cadmium-induced cellular protein aggregation: analyses with stable isotope

Cadmium is a toxic heavy metal preferentially accumulated in the renal cortex of mammals. A possible reason for cadmium cytotoxicity is radical-dependent cellular damage. Ubiquitin-mediated proteolysis has a protective role against cadmium cytotoxicity. We have found that sublethal cadmium exposure induces protein damage to form polyubiquitin-containing aggregates. In the present study, the process of such aggregate formation was analyzed with the method of stable isotope labeling by amino acids in cell culture (SILAC). Sublethal cadmium exposure to a human kidney cell line, HK-2, markedly increases the amounts of polyubiquitin-containing protein aggregates preceding cell death. Cadmium-induced polyubiquitin-containing protein aggregates were similarly formed under the condition of the SILAC experiments. However, content ratios of ¹³C amino acids in both aggregated and nonaggregated protein fractions were changed in the course of cadmium exposure, indicating qualitative alteration of the main component of the aggregates. In addition, cadmium cytotoxicity was enhanced under the condition of SILAC in which a dialyzed fetal bovine serum was employed, suggesting the presence of a factor or factors suppressing cadmium toxicity in the serum.

Ultrastructural analysis of pollen development in a rice cap1 mutant

Collapsed abnormal pollen 1 (CAP1), a novel arabinokinase-like protein, is critical in pollen developments. In mutants, pollen grains lack almost all cytoplasm and collapse. In this study, we used electron microscopy in an attempt to clarify the process of pollen grain degradation. The generative cell wall formed by microspore division presented morphological abnormality, and the nucleus of the cell was condensed and deformed. After that, shrunken protoplast and degenerated intine were observed in the vegetative cell. These results suggest that with a mutant of the CAP1 gene (*cap1*), the cell wall dysplasia of generative cells and the collapse of pollen grains might have been caused by the toxic accumulation of arabinose or by the inhibition of cell wall metabolisms due arabinokinase lacking function.

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 redflanked 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 a 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 excited modes of the ground state have been confirmed to be generated by introducing artificial lattice vibration to a 1-dimensional metallic photonic crystal in which an electromagnetic field between metallic plates is in the ground state. In this study, the effect of optical absorption of metallic material on the radiation of higher excited modes was systematically investigated. We found that the optical absorption inconceivably even enhances the intensity of higher mode radiation and that its optimal values, which maximize the value of peaks in the spectra, exist.

2. Photon-phonon interaction is considered in 1-dimensional disordered metal photonic crystals, which are metal plates of different thicknesses arranged in parallel with an equiinterval. Each metal plate is assumed to be artificially driven. A simple model is numerically analyzed, and the following novel phenomena are discovered. The incident wave with frequencies near the plasma frequency is resonantly amplified. Resonant peaks change with the frequency and the phase of lattice vibration.

3. Topology optimized designs of multiple-disk resonators are newly presented using level-set expression that incorporates surface effects. Effects from total internal reflection at the surfaces of the dielectric disks are precisely simulated by modeling clearly defined dielectric boundaries during topology optimization. The electric field intensity in optimal resonators increases to more than 5 times the initial intensity in a resonant state, whereas in some cases the Q factor increases by a factor of 4 over that for the initial state. Wave-length-scale link structures between neighboring disks improve the performance of the multiple-disk resonators.

4. We have made Monte Carlo simulations to study systems with the ferromagnetic phase transition. Multigrid cluster Monte Carlo method is used to study q=4 Potts models on the square lattices. We calculated relaxation time of order parameters.

Publications

Fujii G¹, Ueta T, Mizuno M¹, Nakamura M² ('Shinshu Univ, ²Akita Pref Univ). Topology optimized multiple-disk resonators obtained using level set expression incorporating surface effects. *Optics Express.* 2015; **23:** 11312-26.

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 the solid state and kinetic probes for dynamic behavior in the solution state.

Research Activities

Synthesis of ¹³C-labeled materials for metabolic and diagnostic research

¹³C-Labeled biologically active compounds are useful as probes for metabolic and diagnostic research because they can be directly applied to mass spectrometry or infrared spectroscopy without separation or purification. We are now engaged in the synthesis of ¹³C-labeled retinol derivatives and ¹³C-labeled biological polyamines. As the new synthetic tools, an automatic liquid-phase synthesizer and an automatic preparative liquid chromatograph were fully utilized.

Exploitation of emissive platinum complexes, exhibiting linkage-length and substitutionposition dependence in emission wavelength

A series of methoxy-substituted trans-bis(salicylaldiminato)platinum(II) complexes incorporating octa-, nona-, deca-, undeca-, dodeca-, and tridecamethylene bridges has been synthesized, and their unique structure-dependencies in the solution-state emission were discovered. Methoxy-substitution on the aromatic rings was found to improve the emission quantum efficiency in the glassy state in 2-methyltetrahydrofuran at 77 kelvin. Chromogenic control over a range of 70 nm was demonstrated, with the 3-, 4-, 5- and 6-methoxy substitutions giving yellow, green, orange, and yellow-green emissions, respectively. These complexes exhibited obvious linker-dependent emission chromism under the same conditions, such that a regular hypsochromic shift from yellow to green

was observed when the linker length was increased.

Publications

Komiya N, Okada M¹, Inoue R¹, Kawamorita S¹, Naota T¹ (¹Osaka Univ). Variations in the emission of polymethylene-vaulted *trans*bis(salicylaldiminato)platinum(II) complexes incorporating methoxy functionalities with linkage length and substitution position. *Polyhedron.* 2015; **98**: 75-83.

Naito M^1 , Inoue R^1 , Iida M^1 , Kuwajima Y^1 , Kawamorita S^1 , Komiya N, Naota T^1 (¹Osaka Univ). Control of metal array based on heterometallic masquerade in heterochiral aggregation of chiral clothespin-shaped complexes. *Chemistry.* 2015; **21:** 12927-39. *Naito M¹*, *Komiya N, Naota T¹ ('Osaka Univ).*

Synthesis, structure and crystal packing of a clothespin-shaped binuclear *trans*-Bis(2-aminotroponato)palladium(II) complex bearing *m*-Xylidene linkers. *J Mol Struct.* 2015; **1102**: 230-4.

Social Science (Law)

Ryuichi Ozawa, Professor

General Summary

Problems of constitutional law in present-day Japan.

Research Activities

OZAWA published 2 Articles and Books from Research Activities 2015 in Japanese.

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

René Descartes' "cogito ergo sum," the ego as a subject of thought, is still a popular and paradigmatic image for the human subject: to be a mature human means that one can think independently and autonomously and one can act according to one's own beliefs. In modern philosophy this image of the ego has been attacked from various positions. One such position, an intersubjective approach, criticizes Descartes' "cogito" as an isolated subject and maintains that an ego can be a subject in only intersubjective relations. Through the recognition of others, one can become and can be a subject. Studies by Don-

ald Winnicott show how important the relationship of the baby with its 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 subject that can think from the universal point of view, as Descartes imagined.

Learn from the experience in Auschwitz

From another respect the "inhuman" situations in the concentration camp Auschwitz show various elements needed to be "human." From the experience written about by Viktor Frankl in Auschwitz we can learn the "human conditions" that in ordinary life remain unconscious but essential.

Japanese

Ikuko Noro, Professor

General Summary

1) A study of the structure of the interview by an experienced psychiatric nurse of patients with mental disease

2) A study of the association among gender, communication, and patient satisfaction

Research Activities

1) We explored the structure of conversation of a well-trained counselor in psychiatric nursing by examining the relations among intention, skill, and function of the statement of the counselor's dialogue with a patient who has schizophrenia and patients with a depression.

2) We held a workshop on gender and communication having Debra Roter, DrPH, of Johns Hopkins University, who is a pioneer in this field, as a keynote speaker, and discussed the associations among gender, communication, and patient satisfaction.

Mathematics

Katsuya Yokoi, Professor

Yasuko Hasegawa, Assistant Professor

General Summary

1. To study dimension theory and topological dynamics

2. To study real analytic automorphic forms and their applications in number theory

Research Activities

1. We studied omega-limit sets, (strong) chain recurrent sets on topological dynamics, and Conley index theory.

2. The construction of automorphic forms has been an important topic in the theory of automorphic forms. We proved the limit formula of the Siegel-Eisenstein series and contributed to it.

Publications

Yokoi K. On strong chain recurrence for 165-77. maps. Annales Polonici Mathematici. 2015; 114:

English

Osamu Ohara, Professor

Tetsuro Fujii, Associate Professor

General Summary

English audio-visual education and digital medieval English study (Ohara) English Language communication and education: material analysis and development (Fujii)

Ohara continued his study of graphology and morphology in the letters of the Stonors in the fifteenth century. Ohara also continued an investigation concerning how to make useful digital images and XML files of fifteenth century manuscripts, especially of the *Stonor Letters*. The results of this investigation were discussed in the papers read at an international conference.

Ohara received a Grant-in-Aid for Scientific Research (C) with 8 other professors in different colleges and began a study concerning the evaluation of students joining the English social networking service community making use of materials from the TED (Technology, Entertainment, Design) Conference.

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

Ohara presented a paper at a session in the 50th International Congress on Medieval Studies held at Western Michigan University in Kalamazoo, MI, USA, in May and also presented the results of his study concerning the graphology of the English medieval letters at Winchester University in November 2015.

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. The revised textbook, *World Trek - English Communication I*, was officially approved by the Ministry in March 2016.

Fujii presented about the effects of teaching materials, exercises, and examinations on students' attitudes toward leaning in "The first year English teaching program that improves leaner motivation" at the 79th Faculty Development Meeting held by The Jikei University in Tokyo in November 2015.

First Foreign Languages

Katsumi Suzuki, Associate Professor

General Summary

German contemporary literature

Research Activities

I am working on the topic of "the modern German literature of nonnative writers in German-speaking areas," especially the works of Ilija Trojanow, who was born in Bulgaria and now lives in Vienna. His novel *The Collector of Worlds* deals with the 3 different worlds of India, Arabia, and Africa. At the academic workshop of the Cross-Lingual Network, I gave a presentation on the topic of "border crossing," which can be found in this novel.

With respect to my working field, at a public lecture organized by the Middle East contemporary literature Study Group, I gave the keynote speech about the trends of the writers in Germany, who had come from Turkey, and the writers of the second and third generation.

Under the concept "What can a literature researcher do in the face of hard facts in Syria," the symposium was organized by the Middle East contemporary literature Study Group and Kyoto University Graduate School of Human and Environmental Studies. I participated as a panelist in it and gave a presentation about Rafik Schami and his latest novel *Sophia oder Der Anfang aller Geschichten*. He is a famous German writer who was born in Syria.

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 measured autonomic nerve activity in healthy persons to develop methods for increasing the comfort of patients with disturbances of consciousness. Satake also reported on the results of a review of the literature on positioning in nursing as an activity of the Japanese Society of Nursing Art and Science, Review Board of Technology Research Results.

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: "Risk factors *of* living with an ostomy *owing to* rectal cancer" and "Health work environment in nursing practice."

Research Activities

Risk factors of living with an ostomy owing to rectal cancer

The aim of this study was to describe the characteristics of living with an ostomy based on the factors of independence in changing appliances and the presence of peristomal irritant dermatitis. Suggestions were made about how stoma clinics can better support the lives of ostomates.

The medcal records of stoma outpatients undergoing twice-weekly stoma care at a hospital were examined. Ostomates who used stoma clinic after formation of a stoma for rectal cancer from January 2008 through July 2014 were surveyed regarding their treatment visit status.

Advanced age and diabetes reduced patients' ability in changing ostomy appliances. Ileostomy and chemotherapy increased the risk of peristomal irritant dermatitis. Most patients with rectal cancer and a stoma were at risk for both or either independence and dermatitis.

Health Work Environment in Nursing practice

About "a follower ship and the leadership" and "possibility of the learning of the manager who utilized experience and the evaluation," I worked on the exchange meeting in the society as a member of the administration staff for the stocktaking for studies.

Adult Nursing

Naomi Takashima, Professor Ruka Seyama, Assistant Professor Yoko Murooka, Assistant Professor Masami Sato, Professor Mai Hosokawa, Assistant Professor Hiromi Nakagawa, Assistant Professor

General Summary

Undergraduate students were offered classroom coursework, including an introduction to clinical nursing and 4 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 and nursing care for acute and critically ill patients.

Based on educational evaluation of the past, the new curriculum was established from the 2012 fiscal year and was offered during the second year of "adult nursing practice theory" for the purpose of critical thinking competency building. We develop class methods further in the first year for care plans for the discharge of surgical patients from the preoperative and postoperative recovery. Teaching methods include group studies based on the problem-based learning of interrelated ways and nursing plans to implement parts of the incorporated planning exercises. Furthermore, practical skills for advanced nursing plans,

collecting information using the video material, and designed thinking in realistic situations, made its own case information paper. Not only were students in a desk planning nursing, but they were deepening the learning in practice as an exercise, heading their agenda, and leading to clinical practice.

Although evaluations by students of nursing process development, which included information collection and nursing practice with nursing plans, were largely positive for the overall adult nursing practicum, evaluations by faculty members tended to be less positive. In the practicum environment and organizational arrangements, the students' cooperation with clinical practicum instructors was strengthened by opportunities for students to review their work with instructors. Students generally gave positive evaluations of educational interventions by faculty members, such as faculty being present at the clinical scene and providing advice and critiques in a timely manner, providing clues on nursing process development based on records, conducting nursing practice with 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 how roles are divided among the parties involved.

Research Activities

Research on Cancer Nursing

1. Research of nursing care for rectal cancer undergoing anterior resection

We have been developing methods of nursing to reduce the bowel disorders typical of anterior resection. This year nurse support program can be obtained certain effects in a study last year that produced for distribution as brochures to more effectively utilize in practice. I product brochures with wound, ostomy, and continence nurse and colorectal surgery doctors, and gastrointestinal oncology nursing researcher. Future plans to study to evaluate the effect of nursing care using the pamphlet with the widely used patient brochures for distribution was created to advance.

2. Research on chemotherapy-induced peripheral neuropathy

By research with other facilities, we have been developing applications for educating patients about chemotherapy-induced peripheral neuropathy. This year, we produced a video that will be delivered by the application. The video teaches patients about how peripheral neuropathy develops and how to deal with it. In addition, a patient assessment tool that was developed the previous year was added to the application so that the patient could use it for self-monitoring. We are now preparing research to evaluate the usefulness of this application.

3. Research on a patient who has cancer and is human immunodeficiency virus-positive For research with other facilities, we have examined the medical treatment of patients who have human immunodeficiency virus and are terminally ill with cancer. A report of such cases was published in the relevant journals. In addition, we have continued this study of human immunodeficiency virus-positive patients for their recognition by infection-control nurses.

Research on a critical care

1. The development of the comfort and palliative care program for patients of the intensive care unit

We examined the stress experienced by patients who received ventilatory support for 24 hours or more in the intensive care unit (ICU). For adult patients without cognitive deficits who were admitted to the general ICU and received ventilatory support for 12 hours or more, the ICU Stressful Experiences Questionnaire was used, and relevant factors were collected from the medical record and analyzed. Data were obtained from 96 patients. Strong stress factors included "thirst of the throat," "the difficulty of carrying on a conversation," and "the displeasure of the endotracheal tube," and relevant factors included the emergent admission and intubation time, a lack in previous diseases, and the total narcotic dose. We would like to propose a nursing support program for the comfort of patients in the ICU and for palliative care and end-of-life care.

2. Analysis of the perioperative pressure ulcer development situation and related factors We are collecting data to prevent the development of perioperative pressure ulcers in patients at high risk because of long-time surgery performed with the patient in a special posture. We are planning to analyze data regarding the pressure ulcer development situation and related factors.

Gerontological Nursing

Fumiko Kajii, Professor

Junko Kusachi, Associate Professor

General Summary

The 4 studies performed in 2015 examined the following topics: (1) the development and evaluation of an application to detect falls and a support program to monitor methods to prevent falls for elderly persons, (2) the development of an educational program to train unemployed and retired nurses to support persons with dementia living in the community and their family caregivers, (3) the effect of seat cushion position on lower-limb edema and blood flow in wheelchair-bound elderly persons requiring seat position support, and (4) the structure of at-home nursing support for elderly persons with dementia, eating disorders, dysphagia, and malnutrition.

Research Activities

Development and evaluation of an application to detect falls and a support program to monitor methods to prevent falls for elderly persons

We performed 4 experiments in this study. First, using a sensor, we estimated the rates of true and false detections of falls during routine activities. Second, we inspected the movement characteristics of transmission and reception between the sensor terminal and server. Third, we assessed whether elderly persons (n = 3) are able to operate smartphones with a

fall-detection application. Finally, we determined if the fall-detection application was able to precisely detect a fall. This work was supported by a Grant-in-Aid for Scientific Research (B).

Development of an educational program to train unemployed and retired nurses to support persons with dementia living in the community and their family caregivers

We performed a mail survey to identify unemployed and retired nurses in a community who were able to benefit from training. We prepared a survey questionnaire to assess the interest in nursing support, the perceived need for nursing support for persons with dementia and their family, and the support activities required to help maintain a work–life balance for nurses. This work was supported by a Grant-in-Aid for Challenging Exploratory Research.

Effect of seat-cushion position on lower-limb edema and blood flow in wheelchair-bound elderly persons requiring seat-position support

We compared the effects of different wheelchair cushions on lower-limb edema and blood flow in elderly subjects with hemiplegia. This work was supported by the Nursing School Research Expenses.

Development of at-home nursing support program for elderly persons with dementia and with eating disorders, dysphagia, and malnutrition

We performed an interview-based study to develop a program for home-visiting nurses to support elderly persons with dementia and with eating disorders, dysphagia, and malnutrition. This work was supported by the Nursing School Research Expenses.

Reviews and Books

Hooper L, Abdelhamid A, Attreed NJ, Cambell WW, Channell AM, Chassagne P, Culp KR, Fletcher SJ, Fortes MB, Fuller N, Gaspar PM, Gilbert DJ, Heathcote AC, Kafri MW, Kajii F, Lindner G, Mack GW, Mentes JC, Merlani P, Needham RA, Olde Rikkert MGM, Perren A, Powers J, Ranson SC, Ritz P, Rowat AM, Sjöstrand F, Smith AC, Stookey JJD, Stotts NA, Thomas DR, Vivanti A, Wakefield BJ, Waldreus N, Walsh NP, Ward S, Potter JF, Hunter P. Clinical symptoms, signs and tests for identification of impending and current water-loss dehydration in older people (Review). Cochran Database of Systematic Reviews. Epub 2015 April 30.

Mental Health and Psychiatric Nursing

Takeshi Katsuki, Professor

Junko Ishikawa, Assistant Professor

General Summary

We give lectures to teach students about medical systems and social resources based on mental health and welfare acts. In addition, we give lectures to teach students methods to assess patients with mental problems on the treatment process.

Research Activities

We have continued to investigate the mental effects of the Great East Japan Earthquake of 2011 on the general population in Japan. Our research is 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 finished analyzing new data in 2015. Significant factors for mental health problems after the Great East Japan Earthquake were clarified as uneasy living in the area with a high risk for the next great earthquake and tsunami disaster, imagining a great deal of damage in the future, and age greater than 65 years with a lack of physical ability.

We presented an interim report at the International Academic Consortium 2016 in Yokohama, Japan.

Moreover, we are researching the human caring approach and have continued to perform discourse analysis.

Child Nursing

Kiyo Hamanaka, Pofessor

Kinu Takahashi, Assosiate 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 then experienced the nursing of children with disease at an acute stage and learned nursing roles in a multidisciplinary team on the practical training for pediatric nursing.

Research Activities

Current situation of nursing ethics and advocacy of children's rights in basic nursing education

Self-administered questionnaires were administered to the teaching staff of 214 educational institutions in Japan. These questionnaires were used to clarify the current situation of nursing ethics and the advocacy of children's rights in basic nursing education. This article was published in the *Japanese Journal of Health Science* (2015; 19(1): 25-30.).

The process of pediatric nurses to achieve practice that advocates for 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 category comprised 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."

The power needed to develop "The Process of Pediatric Nurses to Implement Practice That Advocates Children's Rights"

A strategy was developed to strengthen the ability needed to develop "The Process of Pediatric Nurses to Implement Practice That Advocates Children's Rights."

Three categories affected the intensity of this developed process: "confirmation of children's power," "contrivance to convey children's power," and "attractive emotion to children." There were 6 ways in which the ability to consider children centrally may have been strengthened: "accumulation of experience with children," "accumulation of experience by constantly watching children," "acquisition of knowledge about relationships with children," "accumulation of experience about the relationship with children, with the cooperation of staff," "accumulation of experience on raising consciousness when taking charge of a child," and "fixation and attendance within a location of strength, which facilitates one's ability to consider children centrally." This study was presented at the 11th congress of the Tokyo Women's Medical University Nursing Society in 2015.

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 use of a pessary among patients with pelvic organ prolapse and its effects on daily life and sex life Pelvic organ prolapse (POP) becomes more common in women after childbirth and with aging and is often treated conservatively with a pessary. This study examined patients with POP for whom a pessary was indicated, their symptoms before and after a pessary was instaled, and the effects of the pessary on daily live and sex live. The subjects were 91 patients with POP who were outpatients of the hospital affiliated with University A. Medical records were examined to collect data on the characteristics and symptoms of POP before and after pessary installation. Seven of the patients were interviewed, with their consent, about their daily life and sex life after pessary installation. The 7 patients, whose mean age was 75.8 years, first had POP at a mean age of 68 years. Symptoms before pessary installation included a feeling of pelvic pressure (83.5%), urinary problems (26.4%), and increased vaginal discharge (7.7%). Symptom after pessary installation included increased vaginal discharge (28.6%), urinary problems (24.2%), and bleeding (23.1%). All 7 patients stopped having sexual intercourse after pessary installation because they were concerned that their symptoms would worsen. An effect of POP on the patients' daily life was that at the time of bowel movement they wore thin gloves and pressed against the vagina to keep the pelvic organ inside and that they tried to sit down slowly.

The boundary between discipline and abuse by mothers raising preschool children

This study aimed to analyze and clarify, based on an analysis of narratives regarding parenting behaviors, the boundary between discipline and abuse by mothers raising preschool children. A total of 26 mothers raising preschool children underwent semistructured interviews focusing on the experiences they considered to reflect the boundary between discipline and abuse. Interview transcripts were qualitatively analyzed with a modified grounded theory approach. The 2 categories identified as reflecting aspects related to the boundary between discipline and abuse were "subconscious power of the mother over the child" and "differences in discipline depending on the attributes of the child." Additional categories included "superiority of other people's appraisal of discipline," "maternal idealized image and exhaustion lowering the threshold for discipline," and "external forces and maternal capabilities affecting discipline." Appraisal support from others, provision of knowledge, and instrumental support were found to be important forms of parenting support for mothers.

Comparison of changes in physical indexes of neonatal bathing and dry cleaning care

The purpose of this study was to use stratified randomization, on the basis of sex and body weight, on neonates from 1 day after birth to compare and study objective indices, such as changes in the neonates' body temperature, changes in the number of bacteria, the smell index, and changes in body weight, to investigate the type of cleanliness care of neonates that is regarded as optimal. The subjects of this study were 27 neonates who were born at full term, had no abnormalities at the time of birth, and weighed more than 2,500 g at birth. The neonates were divided into 2 groups: 13 who were bathed in water since the day following birth, and 14 who underwent a dry cleansing from the day after birth. In these neonates, no significant differences were seen in objective indices regardless of whether they had bathed in water or had been given a dry cleansing starting the day after birth.

Community Health Nursing

Junko Shimasawa, Professor Nobuyo Ueda, Assistant Professor Yoshiko Kubo, Assistant Professor Yumiko Shimizu, Assistant Professor

General Summary

The faculty's research has been focused on: (1) visiting nursing care to promote continued community life by mentally ill patients living at home, (2) exploring career anchors among occupational health nurses, (3) reflection among newly-appointed public health nurses in community nursing, (4) health and welfare in patients undergoing hemodialysis who live in the 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 clarity the features of assistance provided by visiting nursing care to promote continued community life by mentally ill persons living at home. In this study, such assistance was considered to be support that promoted continued life in the community of a mentally ill person in a suitable manner.

Exploring career anchor among occupational health nurses

This study examined career anchor characteristics possessed by Japanese occupational health nurses. The data showed the following 5 categories: practices concerning relationships and positions, development of occupational health practices, management skills for effective work, practices approved inside and outside the organization, and considerations of work and private life.

Reflections among newly appointed public health nurses in community nursing

The purpose of this study was to clarify the characteristics to promote reflections among newly appointed public health nurses in community nursing and to thereby obtain suggestions for methods of promoting reflections that enhance their expertise.

Health and welfare in patients who undergo hemodialysis and live in a community

The aim of this study was to examine the problems of health and welfare in patients undergoing hemodialysis who live in a community. This year, we investigated family caregivers and care managers to assess patient's care management.

Nursing care for Directly Observed Treatment, Short-Course

The purpose of this study was to clarity the features of assistance provided by nursing care to patients with tuberculosis who received Directly Observed Treatment, Short-Course, in a hospital.

Home Care Nursing

Motoko Kita, 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

Effectiveness of the flipped classroom in home nursing studies: To ensure knowledge acquisition

We use the flipped classroom to help students develop appropriate assessment viewpoints specific to home nursing. With a view to ensuring knowledge acquisition, we instructed students to review their knowledge with a worksheet after watching movies. As a future approach, it may be necessary to effectively use this method and develop further strategies to learn the theory of home-nursing support.

Processes of discharge support for elderly patients with dementia in acute care hospitals To develop a discharge-support model for patients with dementia in consideration of difficulties specific to the disease, this study aimed to clarify the details of nurses' process of providing such support for elderly patients with dementia in acute care hospitals. The person who should receive support for making treatment decisions for inpatients varied between scheduled and emergency admissions: for scheduled admissions, support for patients was important, and for emergency admissions, support for other family members was important The development of 2 versions of support, for scheduled and emergency admissions, was also found to be essential for establishing a more practical model for discharge support.

Status of information sharing by visiting nurses from the viewpoint of family caregivers In home-based care settings, sharing information with family members is important to provide patients with appropriate care. Family members giving care sought information, in the absence of other family members, about the condition of the person receiving care. Caregivers needed to be informed of the results of assessment, focusing on medical aspects, even if only slight changes are revealed.

Inspection/evaluation

Home Care Nursing has proactively introduced active learning into the class, but further class improvements must be made. We will continue our educational assessment to offer

education that is more effective.

Because all research performed by our instructors involves important subjects in the field of home care nursing, we must support each other and exert our utmost efforts to develop the course.

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