Contents

T /	1	
Intro	dua	etion.
IIIU	aus	JUI OII

The	Continuing Medical Education Committee
Cen	iter for Medical Education
Sch	ool of Medicine
E	Basic Medicine
	Anatomy (Gross Anatomy and Neuroanatomy)
	Anatomy (Histology and Embryology)
	Molecular Physiology
	Cell Physiology
	Biochemistry
	Molecular Biology
	Pharmacology
	Pathology
	Virology
	Bacteriology
	Tropical Medicine
	Public Health and Environmental Medicine
	Forensic Medicine
C	Tinical Medicine
C	Internal Medicine, Division of Gastroenterology and Henatology
	Internal Medicine, Division of Neurology and Trepacougy and
	Internal Medicine, Division of Nephrology and Hypertension
	Internal Medicine Division of Rheumatology
	Internal Medicine Division of Cardiology
	Internal Medicine, Division of Diabetes, Metabolism and Endocrinology
	Internal Medicine, Division of Clinical Oncology/Hematology
	Internal Medicine, Division of Respiratory Diseases
	Internal Medicine, Division of General Medicine
	Psychiatry
	Pediatrics
	Dermatology
	Radiology
	Surgery, Division of Digestive Surgery
	Surgery, Division of Chest Surgery, Breast and Endocrinology Surgery
	Surgery, Division of Pediatric Surgery and Vascular Surgery
	Orthopaedic Surgery
	Neurosurgery
	Plastic and Reconstructive Surgery
	Cardiovascular Surgery
	Obstetrics and Gynecology

Urology	129
Ophthalmology	
Otorhinolarvngology	139
Anesthesiology	
Rehabilitation Medicine	146
Emergency Medicine	
Laboratory Medicine	
Endoscopy	
Infection Control	
Dentistry	167
Transfusion Medicine	171
Department of Molecular Physiology, Division of Physical Fitness	173
Department of Cell Physiology, Division of Aerospace Medicine	175
Department of Pathology, Division of Neuropathology	177
Department of Orthopaedic Surgery, Division of Sports Medicine	179
Department of Allergology	181
Department of Pathophysiology and Therapy in Chronic Kidney Disease	
Research Center for Medical Sciences	
Division of Gene Therapy	187
Division of Oncology	191
Division of Molecular Immunology	194
Division of Medical Engineering	
Division of Neuroscience	199
Division of Clinical Pharmacology and Therapeutics	
Division of Molecular Epidemiology	
Division of Clinical Epidemiology	204
Division of Regenerative Medicine	
Core Research Facilities for Basic Science (Division of Molecular Genetics)	
Core Research Facilities for Basic Science (Division of Molecular Cell Biolog	y)212
Laboratory Animal Facilities	
Radioisotope Research Facilities	
GMP Production Facilities for Cell Therapy and Gene Therapy	
Institute for High Dimensional Medical Imaging	
Institute of Clinical Medicine and Research	
Centers of Advanced Medicine	
Center for Neuroscience of Pain	
Health-Care Center	
Clinical Research Support Center	
Premedical Course	
School of Nursing	

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 a quarter century.

Today, the rapid advances in science and technology provide us with ever greater opportunities to save the lives of patients through research. Japan's rapidly aging society eagerly awaits our research contributions. So that researchers at The Jikei University may join with researchers all over the world to resolve the many pressing health issues, I hope that *Research Activities* plays an increasingly important role.

We owe much to the efforts of Professor Naofumi Kimura, Editor of the Jikeikai Medical Journal, Professor Masao Okazaki, and the members of the Academic Information Center in editing this report.

Senya Matsufuji President The Jikei University School of Medicine

December 15, 2015

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 physicians outside the university hospital. Registered members consist of alumni throughout Japan, members of the local medical association, and physicians 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: 208 (as of April 1, 2015) Members using the Center: 142/year Telephone service: 63 cases
- 2. The 35th summer seminar was held on August 2, 2014. A total of 113 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. Fifteen 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, Associate 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 preparation of a new clinical practice program for fourth-year students starting in 2017.

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 a self-evaluation form as a first step in the accreditation process. Professor Fukushima joined the external evaluation teams as a vice-chair at Niigata University and as a chair at Tokyo Medical and Dental University in 2013 and as a member at the University of Tokyo in 2014. Associate Professor Nakamura joined the external evaluation team of Chiba University in 2014. She published a paper entitled "Quality assurance activity through questionnaire survey for graduates" as a special lecture at Sei-i-kai.

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." In the activity, Associate Professor Nakamura visited King's College London to investigate the final objective structured clinical examination for medical students and the faculty development for general practitioners held at King's College London. Professor Fukushima included dozens of institutions in the elderly in the community for medical care for the elderly program for third-year students starting in 2015.

4. Professor Fukushima attended the Workshop on Experiences of Asia Pacific Countries on the Healthcare Professional Regulation at the 11th International Conference on Medical Regulation on September 10 in London and introduced medical regulation in Japan.

5. Workshop for team-building at a hospital: We organized workshops held in May (Nishi-shimbashi), June (Daisann), July (Kashiwa), September (Nishi-shimbashi), October (Aoto), November (Daisan), December (Kashiwa), and January (Nishi-shimbashi).

6. Contribution to other institutions of higher education (faculty development lectures and workshops): Nara Medical School (April), Showa University School of Medicine (August), IMS Group Patient Safety (Aug), Fukushima Medical College (September), Ishikawa Nursing Center (October), Sapporo Medical College (November), Hyogo Medical School (November), Kawasaki Medical College (December), Showa University (December), Tokyo Medical and Dental University (February), and Toshima Medical Association (March).

Publications

Fukushima O. The quality assurance in basic medical education (in Japanese). *Igaku Shinko*. 2014; **79:** 2-5.

Fukushima O. Fitness to Practice (FtP) activities in U.K (in Japanese). Nippon Naika Gakkai Zasshi.

2014; **103**: 1956-61. *Fukushima O.* The future of pharmacopedia: Education for health care providers (in Japanese). *Farumashia*. 2014; **50**: 1234-6.

Department of Anatomy (Gross Anatomy and Neuroanatomy)

Yoshinori Kawai, Professor

Tohru Hashimoto, Assistant Professor

General Summary

Our department's research activities have focused on neuroanatomy and gross anatomy. In neuroanatomical research, the development and organization of neuronal networks are investigated to elucidate brain function and diseases by means of immunocytochemistry, electron microscopy, in-situ hybridization histochemistry, single-cell tracer injection, and patch-clamp electrophysiology. Our primary interests are the quantitative architecture and dynamics of microcircuits and their relationships. In gross anatomical research, the functional importance of variations in organ systems is investigated with human 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

The region- and size-specific neuronal organizations of the caudal nucleus of the tractus solitarius (cNTS) were investigated, after which excitatory and inhibitory synaptic input patterns onto specific cell types were analyzed by means of patch-clamp recording and immunoelectron microscopy. The 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 gamma-aminobutyric acid-ergic (GABAergic) somata, small calbindin neurons constituted 42% of all cells. The medial subnucleus contained large numbers of glutamatergic, GABAergic, and catecholaminergic somata, and large tyrosine hydroxylase-containing cells constituted 13% of all cells in this region. In total, small neurons (< 150 μ m²) represented 80% of the cell population in the cNTS. Predominant excitatory postsynaptic currents were observed in the adult small neurons, whereas inhibitory postsynaptic currents were more evident in larger neurons, regardless 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 are developmentally established in distinct small and large neurons.

Local axonal arborization patterns of distinct neuronal types in the cNTS

Neurons in the cNTS are heterogeneous in size (50 to 450 μ m² in somal area) and other morphologic characteristics. For a more objective classification of cNTS neurons, their morphologic features were analyzed quantitatively on the basis of reconstructed biocytin-

filled cells after whole-cell patch-clamp recording. According to the pattern of axonal branching behavior, cNTS cells could be classified into 2 groups: smaller cells (mean somal area, 94.1 μ m²; range, 62-120 μ m²; n = 22) and larger cells (mean somal area, 245 μ m²; range, 142-411 μ m²; n = 23). Extensive axonal arborization with numerous possible synaptic boutons was specifically associated with smaller neurons, whereas larger cells possessed few or no axon collaterals, suggesting their distinct roles as local circuit neurons (or interneurons) and projection neurons, respectively. With regard to somatoden-dritic characteristics, the following correlations with cell size were found. Smaller cells had larger form factors than did larger cells (P < 0.05). Larger neurons had more extensive dendritic branching points (P < 0.01), than did smaller cells. These findings suggest that small cNTS neurons contribute specifically to the integration of input information generated in local circuits, whereas large neurons convey the integrated information to other autonomic brain regions.

Postnatal development of GABAergic axon terminals in the rat cNTS

The proper function of the brain depends on a precise arrangement of excitatory and inhibitory synapses. Although the cNTS plays a pivotal role in cardiorespiratory reflexes, we know little about the formation of the local neural network in the cNTS. In the present study, we focused on GABAergic axon terminals and investigated postnatal changes in GABAergic synaptic organizations in the rat cNTS with immunocytochemical studies at both the light and electron microscopic levels. The numbers of synaptic and nonsynaptic GABAergic axon terminals revealed that the number of GABAergic axon terminals in the cNTS was constant until the second postnatal week and that GABAergic axon terminals were reorganized around postnatal day 10 (P10). Electron microscopic observation revealed that most GABAergic axon terminals formed axosomatic synapses on neurons with smaller soma (smaller neurons) at P2 to P4 but that the number of axosomatic synapse decreased considerably after P8. Orphan GABAergic boutons were present specifically near somata of smaller neurons at P10, and the number of axodendritic synapses on thicker dendrites decreased gradually during postnatal development. These results show that GABAergic axon terminals detach from somata of smaller neurons during the second postnatal week. Such morphologic changes in axon terminals could cause changes in electrophysiological activity and might contribute to the reorganization of the local network within the cNTS from the neonatal type to the adult type. These postnatal changes in the cNTS local network might be a prerequisite for the cardiorespiratory reflexes of the adult type.

Activity-dependent reorganization of local circuitry in the developing visceral sensory system

Neural activity during critical periods could fine-tune functional synaptic connections. The activation of *N*-methyl-D-aspartate (NMDA) receptors is critically implicated in this process, and blockade leads to the disruption of normal circuit formation. This phenomenon has been well investigated in several neural systems, including the somatosensory system, but has not yet been evidenced in the visceral sensory system. Ultrastructural

analysis of GABAergic synapses and electrophysiological analysis of inhibitory and excitatory postsynaptic currents of cNTS cells revealed that developmental changes in the synaptic organizations were blocked by MK-801, an NMDA receptor antagonist, when administered at P5 to P8, a presumed critical period for the visceral sensory system. Normal synapse reorganization during postnatal development dictates undifferentiated neonatal cNTS neurons in terms of synaptic input patterns measured with electron microscopy and electrophysiologic studies into 2 cell groups: small cells and large cells under far stronger excitatory and inhibitory influence, respectively. Blockade by MK-801 during the critical period might leave adult neurons wired in the undifferentiated synaptic networks, possibly preventing synapse elimination and subsequent stabilization of the proper wiring.

Glial coverage of small cell somata in the rat cNTS during postnatal development

Astrocytes are thought to be active participants in synaptic plasticity in the developing nervous system. Previous studies have suggested that axosomatic synapses become fewer on the small cells of the rat cNTS toward the end of the first postnatal week. Astrocytes might be involved in this phenomenon. We examined the morphological development of astrocytic processes around the small cell soma in the rat cNTS by means of light and electron microscopy. Structures within the cNTS positive for glial fibrillary acidic protein, glutamate-aspartate transporter, and glutamate transporter 1 became more intensely stained as development proceeded. Glutamate-aspartate transporter-positive structures encompassed calbindin-positive small cell somata after P10. Electron microscopic observations indicated that astrocytic processes as development proceeds. The timing of glial coverage of the small cell soma appears to be consistent with the decrease in axosomatic synapses on the small cells. These observations suggest that astrocytes actively participate in regulating the decrease in axosomatic synapses on small cells in the cNTS during postnatal development.

Quantitative and immunohistochemical analysis of neuronal types in the mouse cNTS: focus on GABAergic neurons

GABAergic neurons are major inhibitory interneurons that are widely distributed in the central nervous system. The cNTS, which plays a key role in respiratory, cardiovascular, and gastrointestinal function, contains GABAergic neurons for regulation of neuronal firing. In the present study, GABAergic neuronal organization was analyzed in relation to the location of subnuclei in the mouse cNTS. On the basis of the differential expression of the messenger RNAs of glutamate decarboxylase (GAD) 67, vesicular glutamate transporter 2, calbindin, and tyrosine hydroxylase (TH), the cNTS was divided into 4 subnuclei: the subpostrema, dorsomedial, commissural, and medial subnuclei. The numerical density and size of somata in the 4 subnuclei were then quantified and analyzed by an unbiased dissector. Calbindin-positive cells constituted subpopulations of small non-GABAergic neurons preferentially localized in the subpostrema subnucleus. The TH-positive cells constituted large neurons preferentially localized in the medial subnucleus. GABAergic neurons constituted a subpopulation of small neurons, preferentially localized in the medial subnucleus.

ized in the commissural and medial subnuclei, which represented at least 50% of small cells in these subnuclei. Thus, the GABAergic small neurons were located around TH-positive large cells in the ventrolateral portion of the cNTS. This finding, in combination with results of previous studies in the rat cNTS showing that large cells originate efferents from the cNTS, suggests that GABAergic small neurons in the commissural and medial subnuclei regulate output from the cNTS.

Postnatal development of axosomatic synapses in the rat NTS: differences between dorsal and ventral subnuclei

Inhibitory axosomatic synapses can effectively suppress the excitability of postsynaptic cells. Examining the development of inhibitory axosomatic synapses is important for understanding the maturation of information processing. The cNTS, which regulates the autonomic system, consists of several subnuclei. In the present study, the development of axosomatic synapses in the dorsal and ventral subnuclei was examined with electron microscopy. In the dorsal subnuclei, the percentage of GAD-positive terminals on the somata, the percentage of small cell somata with synapses, and axosomatic synapse density decreased markedly from P5 to P10. In ventral subnuclei, the percentage of GAD-positive terminals on the soma, the percentage of small or large cell somata with synapses, and axosomatic synapse density were maintained or increased from P5 to P10. Thus, the decrease in inhibitory axosomatic synapses in the dorsal subnuclei might facilitate the maturation of fine receptive areas for peripheral inputs, whereas the increase in inhibitory axosomatic synapses in the ventral subnuclei might facilitate the establishment of an effective regulation system for cNTS output.

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 cNTS, which is generally thought to be homogeneous rather than laminar in cytoarchitecture. By means of in-situ hybridization histochemistry and whole-cell patch-clamp recordings followed by neuronal reconstruction with biocytin filling, the anatomical and functional organization of NTS microcircuitry was quantified to determine associative relationships. Morphologic and chemical features of NTS neurons showed different patterns of process arborization and subnuclear localization according to neuronal type: smaller cells featured presynaptic local axons, and GABAergic cells were aggregated specifically within the ventral NTS. The results suggest both a laminar organization and a spatial heterogeneity of NTS microcircuit connectivity. Geometric analysis of the presynaptic and postsynaptic axodendritic arbor overlap of reconstructed neurons (according to parent somal distance) confirmed a heterogeneity of microcircuit connectivity that could underlie the 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. This finding suggests 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.

Reviews and Books

Arakawa H, Kawai Y. Anatomy and physiology for pernasal endoscopy (in Japanese). In: Suzuki Y, Maruyama M, Obara K, Nito T, Tohara H, editors.

Enge kino hyoka kenshukai tekisuto. Tokyo: PEG Doctors Network; 2014. p. 33-7.

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 evolutionary aspects 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, thus identifying fundamental molecular mechanisms that shape each organ.

Research Activities

Genomic analysis of congenital ataxia mouse

The genomic analysis of the congenital ataxic mouse has revealed the deletion of 7048 bps in the second intron of predicted gene 13912 (Gm13912). The relationship between the ataxic phenotype and genotype of Gm13912 was investigated in the progeny of intercross mice generated by backcrossing B6ICRF1 to C57BL/6N mice 6 times. Most mice showed a clear relationship between the ataxic phenotype and the genotype of Gm13912. However, several phenotypically normal mice showed genotypically ataxic homozygosity for the Gm13912 gene. Interestingly, these phenotypically normal mice also showed genotypically ataxic homo on rs13476689. The other alterations in the genome of ataxic mice are presently under investigation.

Whole exome analysis of Japanese mosaic variegated aneulpoidy syndrome

Mosaic variegated aneuploidy type I (MVAI) is an autosomal recessive disorder characterized by mosaic aneuploidies and involving multiple different chromosomes and tissues. Common clinical manifestations are prenatal onset growth failure, mental retardation, and microcephaly. The cause of the mosaic aneuploidies is the premature onset of anaphase by a defect of the spindle assembly checkpoint (SAC). In several cases cancers or sarcomas or both developed. More than 10 cases of MVAI in Japanese patients have been reported. They showed unique and homogeneous clinical features, such as severe microcephaly and hypoplasia of cerebral hemisphere, Dandy-Walker malformation, and Wilms' tumor. These features suggested that this syndrome contains cilia/centrosome dysfunctions in addition to the SAC dysfunction. Non-Japanese patients with MVAI carry biallelic mutations of the SAC of the BUB1 mitotic checkpoint serine/threonine kinase B gene, BUB1B. All Japanese patients had a nonsense mutation of BUB1B-coding regions on 1 allele but no mutations on the coding region of another allele. Recently, the intergenic single nucleotide mutation at 44 kb upstream from transcription start site of BUB1B was reported as a hypomorphic mutation on another allele. However, the mouse models carrying hypomorphic BUB1B or other SAC gene mutations did not show any brain anomalies or Wilms' tumors. We believed that the hypomorphic mutation could not be explained by clinical features in Japanese cases. Thus, we performed trio whole exome analysis of our case. We found a Japanese-specific nonsynonymous single nucleotide variation of the cilia/centrosome-associated gene on 15q near *BUB1B*. We are evaluating this single nucleotide variation with culture system.

Morphogenesis of the lateral line in the primitive fish Polypterus

Polypterus, the most basal extant actinopterygian fish in molecular phylogeny, possesses ganoid (enamel) scales on the surface of its body, which reminds us of an extinct primitive actinopterygii or teleostei, such as *Psarolepis* or *Lophosteus*. Fossil records of these extinct genera reveal that there are no apparent openings on the surface of the scale for the lateral line neuromast, and in existing bony fishes a wide variety of shapes are known in the lateral line. The lateral line neuromast is the mechanosensory or electrosensory receptor, which is distributed to the cranial and lateral body regions from a part of the cranial nerves. All aquatic animals have the neuromast, despite the lateral line being thought to be a vestigial organ for a terrestrial tetrapod, such as *Xenopus*, that undergoes 3 rounds of whole-genome duplication, as do most actinopterygians. Therefore, we investigated the morphogenesis of the lateral line neuromast in *Polypterus* as a representative model of the primitive actinopterygians.

Initial neuromast cells appeared at neurula as the placode and the neural crest cells to coalesce into apparent rosette structures in the bistratal epidermis, which was observed soon after hatching. At the larval stage, the lateral line neuromasts, hair cells of which bulged out from the surfaces of the epidermides, projected their axons inward lateral line nerve. In a young larva, mineralization starts in a few underlying scales in the rear and advances toward a row of frontal scales. Interestingly, the posterior margin of the lateral line scale transiently appeared, and the neurogenesis appeared to be avoiding calcium deposits for a short period. Scanning electron microscopy of the surface of a juvenile revealed that the epidermis on the lateral line scale had several pores that were nearly covered with mucous substances and were surrounded by the epidermis in a concentric fashion. The neuromasts in the adult fish were macroscopically seen where pigment cells gathered, and the axon bundle from the neuromast threaded the vascular cavities of the lateral line scale inwards and finally pieced it.

Therefore, the lateral line neuromasts along the body existed superficially for life and did not exhibit a distinct feature of the canal structure, even after mineralization, which indicates that the lateral line neuromast in the trunk of *Polypterus* is a superficial pit organ.

Distribution of Wt1-positive cells in diaphragm development

Because the diaphragm consists of several tissues from around the area in which it develops, understanding where the cells come from and which part of the diaphragm they consist of can be difficult. To understand the development of the diaphragm, we focused on the Wilms tumor 1 gene (Wt1), which is related to the congenital diaphragmatic hernia (CDH). We had previously found that Wt1-positive cells are restricted to the left-posterior area of the adult mouse diaphragm. This year, we performed a more detailed analysis of the developmental stages of the mouse embryo and observed an asymmetrical distribution of the Wtl-positive cells more on the left side than on the right side. This finding suggests a relationship between the distribution of Wtl-positive cells and CDH. We will continue to analyze the mechanism of the distribution of Wtl-positive cells to the left side and hope to determine the mechanism of CDH.

Bone formation of vertebrate appendages (limbs and fins)

The paired fins of fish (pectoral and pelvic fins) consist of 2 types of bones, which are derived from lateral plate mesoderm (LPM). In the proximal part of the fin, skeletal elements are formed by endochondral ossification. In the distal part, skeletal elements are formed by intramembranous ossification. To understand the mechanisms of fin bone development, we have established a genome-editing technique with the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system. Several guided (g) RNAs, which bound target sites of the fish genome, were synthesized, but some gRNAs did not work well. However, there were few differences in base sequences in the 3' terminal part. We will collect specimens that form the abnormal skeleton and will raise founder fish and offspring fish to maintain genomeedited fish lines. Moreover, we have established a precise cell transplantation technique for zebrafish embryos by using a micromanipulator. Boxer fish, which are mutants of the exostosin-like glycosyltransferase 3 gene (extl3), do not form the distal part of the fin during embryogenesis. To investigate adult bone phenotypes of boxer fish, boxer LPM cells were transplanted into the wild-type LPM region at gastrulation stages. Boxer LPM cells differentiated, not into LPM derivatives (pectoral fins and heart), but into several mesodermal tissues (blood vessels, muscles and tail structures), because we could not define the host LPM region at earlier embryonic stages. We will improve this transplantation method and will make a fin-specific mutant fish.

Publications

Izuhara L, Tatsumi N, Miyagawa S, Iwai S, Watanabe M, Yamanaka S, Katsuoka Y, Nagashima H, Okano HJ, Yokoo T. Generation of a felinized swine endothelial cell line by expression of feline decay-accelerating factor. *PLoS One.* 2015; **10:** e0117682.

Takeuchi-Igarashi H, Kubota S, Tachibana T, Murakashi E, Takigawa M, Okabe M, Numabe Y. Matrix remodeling response of human periodontal tissue cells toward fibrosis upon nicotine exposure. Odontology. 2014 Oct 15. Epub ahead of print.

Reviews and Books

Yano T, Matsubara H^I, Egawa S^I, Onodera K^I, Tamura K^I (^ITohoku Univ). Chapter 22: Fins and limbs: emergence of morphological differences. In: Kondoh H, Kuroiwa A, editors. New principles in developmental processes. Tokyo: Springer; 2014. p. 291–302.

Department of Molecular Physiology

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

General Summary

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

Research Activities

Differential scanning calorimetry measurement of water components in skinned skeletal muscles

Magnetic resonance images reflect both water content and water states in tissue. Recently, taking advantage of well-organized skeletal muscle, we clarified that magnetic resonance distinguished 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, is believed to restrict their mutual motional freedom to result in one of the water states. Therefore, 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 changes of skinned fibers with or without thick filament to find the extra heat absorption with temperature. There were endothermic peaks at 50°C and 65°C in a temperature-dependent irreversible manner. The denaturing peaks at approximately 50°C were affected by the presence of thick filament, but those at approximately 65°C were not. Additionally, we found 2 significant endothermic peaks at $< -10^{\circ}$ C and at about the melting point of water. The peaks at about -21° C were affected by the presence of thick filament, whereas the peaks at about -23° C were not. Accumulated enthalpy as an index of overall heat capacity was affected by the presence of thick filament. These results suggest that peaks at -21° C and 50° C originate from mainly thick filament and that peaks at -23° C and 65° C originate from mainly thin filament.

Viscoelastic property evaluated with quartz crystal microbalance

We observed the adsorption process of myosin to a gold surface with a quartz crystal microbalance. Viscoelastic properties of myosin adsorbed to the surface of the gold electrode and its surrounding solution as a whole were studied with a quartz crystal microbalance molecular interaction analyzer (AFFINIX QN Pro, Initium, Tokyo). Samples were measured in both solution and air. The adsorbed protein volume was calculated from the data in the air.

When myosin was adsorbed more sparsely than 0.2 μ g/cm², the viscoelastic change accompanying the myosin adsorption was almost the same as the viscoelasticity of buffer without myosin. As the weight of the adsorbed myosin decreased, the resonance fre-

quency decreased. This finding suggests that myosin adsorbed at a low density acts as a solid globular protein. On the other hand, when myosin was adsorbed at a higher density, large viscoelastic change was observed. Viscoelastic analysis indicates that myosin plays as a protein having viscoelasticity and that binding ATP to the head of myosin changes the viscoelasticity of the protein. This suggests that when adsorbed closely, myosin immobilizes the surrounding solution. Half of this immobilized solution was released in the presence of ATP or ADP but not in the presence of ATP- γ S.

Chlorogenic acid, a polyphenol in coffee, protects neurons against glutamate neurotoxicity

The study was designed to explore the molecular mechanism of chlorogenic acid (CGA) in protecting against glutamate-induced neuronal cell death. We investigated the protective effects of CGA on glutamate-induced neuronal cell death using primary cultures of mouse cerebral cortex because the release of glutamate during brain ischemia triggers the deaths of neurons. Cortical neurons in primary culture were exposed to 300 µM L-glutamic acid or vehicle, with or without 10 µM CGA or 10 µM MK-801. After 16 hours, primary cultures were stained with propidium iodide/Hoechst or calcein. Double-staining with propidium iodide and Hoechst was performed to confirm whether the cell death induced by glutamate was apoptotic. Intracellular concentrations of Ca²⁺ were observed with the Ca²⁺-indicator fura-2. Glutamate-induced neuronal cell death was inhibited by treatment with CGA. In addition, CGA prevented the increase in the intracellular concentration of Ca^{2+} caused by the addition of glutamate to cultured neurons. On the other hand, CGA had little effect on cell death induced by nitric oxide, which is downstream of the ischemic neuronal cell death. Our results suggest that the polyphenol CGA in coffee protects neurons from glutamate neurotoxicity by regulating the entry of Ca^{2+} into neurons. Therefore, CGA in coffee may have clinical benefits for neurodegenerative diseases, such as ischemic stroke.

Intrinsic structural change of helically arranged myosin heads in skeletal muscle sarcomere in the absence of thin filaments

Myosin converts the chemical energy of ATP to mechanical work in combination with actin. The molecular mechanism of this chemomechanical transduction is still unknown, mainly because mechanical work significantly deforms the molecules. Therefore, it is of interest to follow the intrinsic structural changes of myosin in the absence of actin.

We followed intrinsic structural changes of myosin heads in sarcomeres, where conformational freedom of myosin would be highly restricted in a range optimized for the physiological path, unlike in the purified solution system.

Actin was removed from sarcomeres of skinned fibers with gelsolin treatment, and helically arranged myosin heads were observed with X-ray diffraction (at BL6A of PF) following the ATP hydrolysis steps of M, M-ATP, M-ADP-Pi, M-ADP, and M, in which "M" represents myosin. Compared with M and M-ATP (trapped with N-phenylmaleimide) states, myosin heads in the M-ADP-Pi state was retracted close to the backbone of the thick filament. Retrograde binding of ADP to M to yield M-ADP did not cause this marked transition of myosin heads. Because the orthograde conformational change in the M-ADP state following Pi release is generally considered to be coupled with the mechanical work of myosin, radial retraction and the following protrusion of myosin heads would likely be the prime mover, as in the case of crawling bristle grass in your gripping hand.

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). In addition, we developed an *in vivo* nanoimaging system to observe sarcomere contraction in the ventricles of small animals, such as the rat and mouse.

Research Activities

Development and pathogenesis of the great arteries

1. Molecular mechanism of closure of the DA

The DA is a mysterious artery that is interesting to study. The DA is an essential vascular shunt between the aortic arch and the pulmonary trunk during fetal development. The DA closes immediately after birth in accordance with its smooth muscle contraction and vascular remodeling. When the DA fails to close after birth, the condition is known as patent DA, which is a common problem in premature infants. Although cyclooxygenase inhibitors are often used to treat patent DA, their efficacy is often limited. Both vascular contraction and remodeling, i.e., intimal thickening, are required for complete anatomical closure of the DA. Decreased elastogenesis is a hallmark of DA remodeling 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 prostaglandin E_2 (PGE₂) receptor EP4 signaling promotes degradation of the mature lysyl oxidase protein, a cross-linking enzyme for elastic fibers, only in the DA, leading to decreased elastogenesis. We also found that phospholipase C, but not phosphokinase A is involved in EP4mediated degradation of the mature lysyl oxidase protein. In addition, we found that thromboxane A2 plays a role in both vasoconstriction and the promotion of vascular remodeling in the rat DA. Furthermore, using DNA microarray analyses, we examined the transcriptional profiles of the DA in the Brown-Norway rat, which often exhibits patent DA. We newly identified more than 70 DA-dominant genes that may play an important role in DA-specific functional and morphologic characteristics.

2. Molecular mechanism of elastic fiber formation in the great arteries

Abdominal aortic aneurysm (AAA) is a common but life-threatening disease among the elderly. In collaboration with Yokohama City University, we developed smooth muscle cell-derived 3-dimensional multilayers as a new experimental model for vascular elastic fiber formation studies.

Regulation of cardiac sarcoplasmic reticulum ATPase activity

Impaired Ca²⁺ 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 Ca²⁺-ATPase and Ca²⁺ homeostasis in the sarcoplasmic reticulum. Using 2 types of transgenic mice that exhibited either sarcoplasmic reticulum Ca²⁺ overload or Ca²⁺ deficiency, the selective modulation of the sarcoplasmic reticulum Ca²⁺-ATPase activity did not change Ca²⁺ leak from the sarcoplasmic reticulum. Our data indicate that Ca²⁺ leak is independent of sarcoplasmic reticulum Ca²⁺ uptake.

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. It has been known that the main cardiac metabolism switches from fatty acid oxidation to glycolysis when the heart has stress. Using capillary electrophoresis and mass spectrometry, we are investigating the key metabolite(s) or enzyme for this switch when the heart begins to fail.

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 spatial 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. First, 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 marked underestimation of sarcomere lengthening speed due to the superposition of different timings for lengthening

between sequentially connected sarcomeres. Then, we found that following treatment with ionomycin, neonatal myocytes exhibited spontaneous sarcomeric oscillations (Cell-SPOC) at partial activation with blockage of sarcoplasmic reticulum functions and that the waveform properties were indistinguishable from those obtained in electric field stimulation. The present experimental system has a broad range of possible applications for unveiling single sarcomere dynamics during excitation-contraction coupling in cardiomy-ocytes 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

Tadashi Asakura, Associate Professor

General Summary

Tumors are 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

Investigation of new regulators that function in the development and maintenance of the breast cancer stem cells

Cancer stem cells have been suggested to compel tumor development and progression. Although breast cancer stem cells were discovered in 2003, the molecular mechanism of their development and maintenance remains poorly understood. To explore the new regulators that function in the development and maintenance of breast cancer stem cells, we first used flow cytometry to examine cancer stem cells population (markers: CD44⁺/CD24⁻ and aldehyde dehydrogenase [ALDH]) in triple-negative breast cancer cell lines. Unexpectedly, the percentages of cells marked with CD44⁺/CD24⁻ and ALDH were inconsistent in the different cell lines we used. Therefore, cancer stem cell populations are difficult to separate with known markers, such as CD44⁺/CD24⁻ and ALDH. Thus, for the further experiments we have made cancer stem cell-enriched populations with the sphere culture method.

Discovery of the molecular mechanism of metastasis in breast cancer stem cells

A line of breast cancer stem cells, iCSCL-10A, was established in 2014 by introducing defined reprogramming factors (OCT4, SOX2, Klf4, and c-Myc) into MCF-10A nontumorigenic mammary epithelial cells. The iCSCL-10A cells possess the hallmarks of cancer stem cells and develop tumors in immunosuppressed mice. However, the metastatic ability of iCSCL-10A cells is unknown. We examined, with an in-vivo imaging system, the metastatic ability of iCSCL-10A cells that overexpressed red fluorescent protein E2-Crimson in immunosuppressed mice. Whereas no metastasis developed in mice to which control MCF-10A cells had been injected, bone metastasis developed near the femur, tibia, and spine after 6 weeks in mice to with iCSCL-10A cells had been injected. In a further experiment, the molecular mechanism of metastasis will be examined by

comparing those metastasized cells with iCSCL-10A cells.

Generation of DYRK2 knockout mice

We have previously shown that dual specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2) phosphorylates tumor suppressor p53 and transcription factors, c-Jun and c-Myc. The dysfunction of DYRK2 promotes tumor-formation activity in a xenograft model. However, the in-vivo role of DYRK2 is unknown. To investigate the in-vivo function of DYRK2, we generated knockout mice lacking the DYRK2 gene (*DYRK2*) by clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) genome-editing system. Synthetic RNA targeting *DYRK2* and Cas9 nuclease were coinjected into fertilized eggs. As a result, efficient production of mice that carry biallelic mutations in a given gene were created. To determine the mutations generated by CRISPR/Cas9, we examined the sequence of exon 3 of *DYRK2* from 13 pups. Despite no biallelic frameshift mutations being found in all founders, we observed single allelic frameshift and nonsense mutations in 5 founders. At the present time, *DYRK2*^{+/-} heterozygotes have been crossed to obtain *DYRK2*^{-/-} homozygotes, *DYRK2*^{+/-} heterozygotes, and wild-type littermates.

Production of DYRK2 gene-targeted embryonic stem cells

Insights from cell-based research suggest that DYRK2 has tumor suppressive functions. To study the physiological and pathological roles of DYRK2, we attempted to conditional knockout of the gene in mouse. The targeting vectors, obtained from the Knock-out Mouse Project, were checked by restriction enzyme digestion and the polymerase chain reaction. Linearized targeting vector was introduced into embryonic stem (ES) cells by electroporation. After extracting the genome DNA from drug-resistant ES clones, we screened for correctly targeted events with the long-range polymerase chain reaction and Southern blotting. Selected ES cell clones show the correctly recombined *DYRK2* allele, which is the knockout-first allele (tm1a) that can be used for the generation of reporter knockouts, conditional knockouts, and lacZ-tagged null alleles following exposure to site-specific recombinases Cre and Flp.

DYRK2 and cancer stem cells

Our recent study revealed that DYRK2 has a tumor-suppressive function through expression of c-Myc, c-Jun, and Snail and phosphorylation of p53. The expression of DYRK2 is decreased in advanced breast cancer and serous ovarian cancer. Diminished expression of DYRK2 confers drug-resistance to cytotoxic chemotherapy and poor prognosis in these cancers. However, the therapeutic strategy has not been established for patients with breast cancer and low DYRK2 expression. Through microarray analysis, a mechanistic target of rapamycin (mTOR) complex 1 pathway was detected as the activated pathway in DYRK2-depleted cells. Treatment with everolimus, an mTOR inhibitor, was associated with a significant inhibition of tumor growth compared with a vehicle in vitro and in vivo.

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

was upregulated and resulted in an increased production of cancer stem cells. We will next analyze the association between DYRK2 and cancer stem cells in clinical samples.

Monopolar spindle 1 kinase regulates mitotic chromosome condensation

In all organisms, the control of cell-cycle progression is a fundamental process for proliferation, development, and survival. During each of phase the cell cycle, the expression of numerous proteins and the status of their posttranscriptional modifications are dramatically changed. An important modification during mitosis is that of phosphorylation. We focused on monopolar spindle 1 (Mps1), a well-conserved protein kinase that is an essential regulator for proper mitotic progressions. To identify proteins associated with Mps1 during mitosis, we performed mass spectrometry analysis. We identified condensin II as a novel Mps1-associated protein. Condensin is a highly conserved complex that contributes to mitotic chromosome condensation and segregation. The condensin complex is pentamer, which are composed of structural maintenance of chromosomes (SMC) heterodimers (SMC2 and SMC4) and non-SMC subunits (chromosome-associated protein [CAP]-H2, CAP-G2, and CAP-D3). We performed further analysis and revealed that Mps1 phosphorylates Ser492 of CAP-H2 during mitosis. Silencing of Mps1 inhibits Ser492 phosphorylation and the chromosomal recruitment of condensin II at mitosis. Furthermore, knock-down of Mps1 disrupts the chromosome condensation in prophase. These results suggest that Mps1 affects chromosomal condensation in the early phase of mitosis by regulating condensin II.

Novel cancer-associated protein thyroid hormone receptor interacting protein 13

In yeast, pachytene checkpoint 2 (Pch2) regulates several meiotic processes, such as synaptonemal complex formation, interhomologous recombination, and DNA double-strand breaks repair. Thyroid hormone receptor interacting protein 13 (TRIP13) is the mammalian ortholog of yeast Pch2. The expression profile of TRIP13 is highly restricted and most abundant in the testis but is aberrantly expressed in several types of cancers. We performed Western blotting to detect the expression levels of TRIP13 among 12 human cancer cell lines and 2 immortalized cell lines. This analysis revealed that all of the cell lines expressed high levels of TRIP13. Interestingly, TRIP13 expression was upregulated in an immortalized lung fibroblast cell line, WI-38 VA13 subline 2RA, as compared with parental normal fibroblast cells. In addition, TRIP13 was phosphorylated during the M phase in synchronized HeLa cells. These finding suggests that TRIP13 has potentially important roles in cell proliferation and tumor growth.

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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 a feedback mechanism involving AZ. The AZs are further regulated by proteins termed antizyme 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

Role of AZ2 in c-MYC degradation

We have previously identified AZ2 as a c-MYC-associating protein that colocalizes with c-MYC in the nucleus and nucleolus and accelerates c-MYC degradation by the proteasome in an antizyme-dependent and ubiquitin-independent manner. We successively elucidated the effects of AZ2 on c-MYC degradation in the nucleolus. The overexpression of the nucleolar protein nucleophosmin 1 (NPM1) has been reported to increase the nucleolar localization of c-MYC and the rate of its degradation. Under this condition, we investigated the effects of knockdown and overexpression of AZ2 on c-MYC degradation in the cells. The knockdown of AZ2 suppressed degradation of endogenous c-MYC. In contrast, overexpression of AZ2 increased the rate of degradation of both wild-type c-MYC and a nonubiquinated mutant of c-MYC (T58A), which can be degraded in the presence of NPM1. These results suggest that AZ2 accelerates the degradation of c-MYC in the nucleolus in an ubiquitin-independent manner.

Analysis of interaction between AZ2 and ATP citrate lyase

A cytoplasmic enzyme, ATP citrate lyase (ACLY), generates acetyl-coenzyme A from mitochondria-derived citrate and is important for fatty acid synthesis and histone acylation. We have previously identified ACLY as an AZ2-interacting protein and showed that ACLY can interact with both AZ1 and AZ2 and that AZs accelerate ACLY activity *in vitro*. We further investigated mechanism for activation of ACLY by AZs. In cells, induction of AZs by polyamines increased ACLY activity, whereas knockdown of AZ suppressed ACLY activity. The enzyme ACLY is known to be activated by Ser455 phosphorylation, but AZs did not change the phosphorylation at this residue. These results indicate that AZs activate ACLY through novel mechanisms. We are also developing a method for measuring ACLY activity with mass spectrometry and stable isotope-labeled citrate.

Characterization of hematopoietic stem cells in AZ1 knockout mice

We previously showed that the knockout of the gene *AZ1* in mouse increases tissue polyamine levels, reduces the numbers of hematopoietic progenitor cells of multiple lineages (erythroid, granulocyte-monocyte, and common myeloid), and possibly increases the number of hematopoietic stem cells (HSCs) with lowered activity. To discriminate whether the activity of HSCs are generally decreased or only a subpopulation of HSCs are affected, we measured the activity of individual HSCs with limited dilution in transplantations in which the number of donor fetal liver cells per recipient animal was reduced as much as possible. Four months after the transplantation, the reconstituted peripheral blood cells were analyzed in mice engrafted with $AZ1^{+/+}$ HSCs or $AZ1^{-/-}$ HSCs. Some $AZ1^{-/-}$ HSCs-engrafted recipients exhibited a HSC multipotency comparable to that in $AZ1^{+/+}$ HSCs-engrafted recipients, while the other recipients barely had the myeloid progeny from $AZ1^{-/-}$ HSCs. These results indicate heterogeneity in the multipotency of the $AZ1^{-/-}$ HSC pool.

Analyses of physiological roles of Azin1

To address the regulatory roles of Azin1, we analyzed mouse embryonic fibroblasts (MEFs) from a mutant mouse in which expression of Azin1 is greatly diminished. It was noted that the mutant MEF contained many small-shaped cells (\sim 30% of total cells). The activity of ODC in G2/M-synchronized mutant MEFs was only 3.3% of that in the wild-type MEFs, and that induced by cell dilution in the mutant MEFs was 23% of that in the wild-type MEFs. These results suggest the physiological importance of Azin1 for normal cell growth. Next, we compared the expression pattern of proteins in the 2 MEFs. We found some interesting proteins, including ACLY, being differentially expressed. We also analyzed metabolite profiling of the 2 MEFs by capillary electrophoresis-mass spectrometry. Strong metabolic abnormality in polyamine and methionine metabolism was noted in the mutant MEFs.

Analysis of AZ frameshifting mechanism with human PURE system

The human PURE system is an *in vitro* translation system reconstituted with purified human translational factors. A merit of this system is the ease with which specific factors, such as polyamines, can be added or removed or both. Using this system, we are analyzing the molecular mechanism of ribosomal frameshifting of AZ expression. A frameshift product of human AZ1 was synthesized in the system in a polyamine-dependent manner, although the efficiency was still low. We started several experiments in the system to reveal the cis-acting elements or trans-acting factors or both participating in AZ frameshifting.

Interaction analysis of spermine-binding RNA aptamers with spermine

Aptamers of RNA have the potential for both clinical and research applications. In partic-

ular, aptamers are useful for exploring RNA-binding sequences and structures for target molecules. We are revealing general polyamine-binding RNA sequences and structures by analyzing polyamine-binding sites on isolated RNA aptamers. We have isolated a spermine-binding aptamer. To improve the data of binding affinity last year, we continued interaction analyses using isothermal titration calorimetry and detected the specific 1:0.85 interaction between spermine and this aptamer. With this result, the dissociation constant was estimated to be 27.2 μ M. Furthermore, resonances of imino protons in basepairs and H5-H6 cross-peaks of pyrimidine were monitored with nuclear magnetic resonance spectroscopy to investigate the effect of spermine on the tertiary structure of the aptamer. In the presence of spermine, several resonances in these spectra had disappeared or shifted. Interestingly, the affected bases by spermine were positioned on a wide range in this aptamer. We further investigated a thermal stability of the aptamer with or without spermine by UV melting. The melting temperature was shifted to a higher degree in the presence of spermine. These results suggest that spermine induces a dynamic conformational change of this aptamer and stabilizes the aptamer-spermine complex.

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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. Intracellular functions of endozepine (Yuji Ohno)

4. Study of purinergic receptors in human-derived mast cells (Haruhisa Nishi)

5. Significance of cerebellar sagittal zones in cerebrocerebellar communication (Taro Ishikawa, Misa Shimuta)

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

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. Electrochemical analyses were also performed with a new biosensor material, carbon nanotube, to elucidate the mechanisms of catecholamine release in the midbrain. Furthermore, optogenetic activation techniques for neurones in these brain areas are being introduced to analyze local neural circuits.

Another issue is the regeneration of synapses and local circuits after basal ganglia-related disorders. Electrophysiological, morphological, and behavioral studies were performed to elucidate the mechanisms and time course of the reconstruction of synaptic organization and transmission and the functions of whole animals in Parkinson's disease model rats. In addition, the function of physiologically released dopamine has been analyzed in the regulation of synaptic transmission as well as in behavior, using dopamine receptor knock-out mice.

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

Yawning in mammals has been considered a respiration-related behavior because of the accompanying long-lasting inspiration and brief expiration. However, aquatic turtles, amphibians, and air-breathing fishes with lungs, unlike mammals, never open their glottis during yawning. Sharks, which lack lungs, also show yawning-like behavior. "Yawning" in the sharks is characterized by their stretching their jaws (considered to be derived from the first gill arch) and the remaining gill arches. An act similar to yawning in sharks was examined in more primitive jawless fish, the lamprey. Lampreys, when they stopped sucking the wall of the tank, occasionally stretched their branchial arches and the rostral part of their bodies. "Yawning" in vertebrates may be redefined as a stretching movement of the branchial arches or of derived structures (such as the jaw and pharyngolarynx).

Intracellular functions of endozepine

In the central nervous system, endozepine is known as an endogenous anxiogenic peptide that can suppress GABA binding to GABA_A receptor through the association of the peptide with a benzodiazepine receptor. In addition to this extracellular or intercellular function, we have suggested, in bovine adrenocortical cells, that the peptide could promote steroidogenesis through intramitochondrial cholesterol transport. To obtain the protein, we extracted the messenger RNA from bovine adrenocortical cells and the complementary DNA of endozepine was amplified with the polymerase chain reaction to insert in expression vectors of *Escherichia coli*. However, the peptide expressed by *E. coli* could not exert sufficient function. In view of posttranslational modification including glycosylation, it should be expressed by mammalian cells, such as HEK 293. After the isolation and purification of endozepine, we would like to examine whether the protein could enhance steroidogenesis in adrenocortical mitochondria.

Study of purinergic receptors in human-derived mast cells

The role of purinergic receptors in mast cell degranulation was investigated in leukocyte adhesion deficiency 2 cells, a human-derived mast cell line. Particular attention was focused on the purinergic receptors for $P2Y_{11}$ and $P2Y_{14}$ and their properties. Our findings are as follows:

1. Stimulation of mast cells by adenosine 5'-O-(3-thio)triphosphate (ATP γ S), a P2Y₁₁selective agonist, induced both intracellular Ca²⁺ mobilization and phosphorylation of phosphatidylinositol 3-kinase (PI3K) and Akt. Stimulation of ATP γ S also resulted in synergistic enhancement of allergic degranulation following stimulation of Fc epsilon receptor I, a high-affinity immunoglobulin E receptor, while the agonist did not induce degranulation by itself.

2. Uridine diphosphate-glucose, a $P2Y_{14}$ -selective agonist, did not induce Ca^{2+} mobilization, and neither PI3K nor Akt phosphorylation was observed. However, the agonist did induce degranulation without any allergic stimulation of the cells.

These results show that mast cells express multiple purinergic receptor subtypes linked to degranulation via different intracellular pathways. These findings show the feasibility of targeting distinct purinergic receptors on mast cells as part of therapeutic strategies to reduce allergic symptoms.

This study was presented at the 88th Annual Meeting of the Pharmacological Society (Nagoya, March 2015).

Significance of cerebellar sagittal zones in the cerebrocerebellar communication

The cerebellar cortex receives descending signals from the cerebral cortex as well as sensory signals from the periphery. However, how these 2 pathways of inputs are integrated and the role of such interaction are poorly understood. Moreover, it is unclear if input pathways are different for individual sagittal zones, which are recognized by the expression of a glycolytic enzyme aldolase C, in the cerebellar cortex. To address these issues, we used knock-in mice that express Venus fluorescent protein in aldolase C-positive cells (provided by Professor Izumi Sugihara, Tokyo Medical and Dental University). After visually indentifying the sagittal zones in these mice, we simultaneously recorded field potentials from the cerebellar granule cell layer in crus II and the cerebral somatosensory cortex in anesthetized mice. We found that the cerebellar response has biphasic peaks and that the first and second peaks corresponded to the direct pathway from the peripheral and the indirect pathway from the cerebral cortex, respectively. We also found that the direct signal was strongest in the aldolase 5 - band and that the ratio of the indirect signal was highest in the most lateral 7+ band. Furthermore, we made whole-cell patch-clamp recordings from individual granule cells in vivo and found that some granule cells have biphasic excitatory postsynaptic currents, indicating that mossy fiber inputs from 2 pathways converge onto single granule cells. On the basis of these findings, we are conducting research to clarify the mechanism of cerebrocerebellar communication.

The basic mechanism underlying the anticonvulsant effects of a ketogenic diet

A ketogenic (low-carbohydrate, high-fat) diet has been used successfully to treat pediatric and medically refractory epilepsy. The mechanisms underlying the success of ketogenic diet therapy, however, are not well understood. To elucidate these mechanisms, we used a complementary approach that included in vivo dietary treatment followed by the electrophysiological characterization of acute brain slices. We fed rats and mice a ketogenic diet or a control diet for 2 to 3 weeks, prepared acute hippocampal slices, and performed electrophysiological and pharmacological studies in the seizure-prone CA3 region of the hippocampus. Slices from animals fed a ketogenic diet showed reduced excitability, and seizure propensity depended on maintaining a reduced extracellular glucose level. This reduced excitability was not observed in rats and mice fed a control diet. The effects of the ketogenic diet could be reversed with blockers of adenosine A_1 receptors and were absent in slices obtained from mice lacking adenosine A_1 receptors fed a ketogenic diet. These results suggest that the reduction of neuronal activity through activation of adenosine A_1 receptors is a key mechanism underlying the anticonvulsant effects of a ketogenic diet.

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Department of Pathology

Masahiro Ikegami, Professor Akihiko Sakata, Professor Takako Kiyokawa, Professor Hiroyuki Takahashi, Associate Professor Yukiko Kanetsuna, Assistant Professor Tohru Harada, Assistant Professor Tomoe Lu, Assistant Professor Masaharu Fukunaga, Professor Masafumi Suzuki, Professor Satoru Chiba, Associate Professor Koichi Nomura, Associate Professor Yasuhiko Endo, Assistant Professor Shigeharu Hamatani, Assistant Professor Masakazu Komine, Assistant Professor

Research Activities

Liver research

1. Primary biliary cirrhosis was newly classified with Nakanuma's classification, and biochemical data and histopathological findings were compared. Changes in the histopathological findings of hepatitis, cholangitis, bile duct loss, and fibrosis vary among patients, and cases have been found with improvement, no change, or progression of each finding. Bile duct loss and fibrosis represent important histopathological factors in Nakanuma's classification. Various findings of improvement, no change, and progression are also observed in each stage according to Nakanuma's classification. Moreover, because some patients showed improvement of inflammation but progression of bile duct loss, a worse prognosis was feared. The changes in clinical findings do not correspond with changes in histopathological findings. In other words, histopathological findings of inflammation and chronic nonsuppurative destructive cholangitis are sometimes seen even in patients with improved values of alkaline phosphatase and γ -glutamyl transpeptidase. The follow up of patients with primary biliary cirrhosis should, therefore, include both biochemistry laboratory tests and liver biopsy for histopathology.

2. Changes in the liver with aging: Imaging was performed for liver tissue samples from 45 autopsy cases. The size of each hepatocyte was compared with the sizes of the sinusoids and spaces of Disse to investigate changes associated with aging. The sizes of the sinusoids and the spaces of Disse relative to each hepatocyte decreased with aging, and hepatocytes became slightly larger. These findings suggest sinusoid shrinkage and a stromal increase with age.

3. Central necrosis in autoimmune hepatitis was examined in liver biopsy specimens from 72 patients. Forty-eight patients showed centrilobular necrosis and inflammation, 33 had centrilobular necrosis, and 45 had central inflammation. Among the patients with central inflammation, 23 had extension of interface hepatitis and 22 had an opposite expansion of inflammation from the centrilobular areas to the peripheral liver parenchyma. Centrilobular necrosis is not uncommon in autoimmune hepatitis, and a so-called "opposite interface hepatitis," involving the centrifugal spread of inflammation from the centrilobular areas, was a notable finding.

Gastrointestinal research

1. Pathology of colorectal neuroendocrine tumors: Under the World Health Organization

Classification of Tumors of the Digestive System established in 2010, colorectal neuroendocrine tumors have been classified on the basis of the mitotic count and the Ki-67 index into neuroendocrine tumors grades 1 and 2 and neuroendocrine cell carcinoma. With this change, patients previously receiving diagnoses of carcinoid tumors at our hospital were reclassified, and the correlations with vascular invasion and prognosis were examined. A total of 115 patients with colorectal carcinoid tumors were treated at our hospital, with endoscopic resection in 98 patients and surgical resection in 17 patients. Among these 115 patients, 95 (82.6%) had G1 lesions and 20 (17.4%) had G2 lesions. Among the 17 patients undergoing surgical resection, 10 (58.8%) had G1 lesions and 7 (41.2%) had G2 lesions. Seven (41.2%) of the patients undergoing surgical resection had lymph node metastases. Metastases were more common with G2 lesions (5 patients, 71.4%) than with G1 lesions (2 patients, 20%). All patients with metastases showed vascular invasion, suggesting vascular invasion as an important risk factor for metastases.

2. Objective indices for colorectal serrated lesions: We aimed to create objective indices for colorectal serrated lesions (CRSLs) by classify CRSLs with objective indices we have developed and by comparing rates of gene mutation and phenotypic expression. We examined whether the sizes of the nuclei of epithelial cells decreased from the lower part to the surface of serrated gland ducts (an index of cell maturity) and whether gland duct branching (an index of complexity of gland duct structure) was present. On the basis of these 2 objective indices, we morphometrically verified that CRSLs can be classified into 5 types.

3. Clinicopathological features of duodenal epithelial tumors: Duodenal epithelial tumors have not been classified histologically. We therefore classified duodenal epithelial tumors on the basis of the immunohistologic examination of their mucinous characteristics and investigated their clinicopathologic features. We examined 110 duodenal epithelial tumors that were surgically or endoscopically resected at our hospital. Staining with hematoxylin and eosin and various immunostaining tests were performed on the samples to classify the lesions as an intestinal type or gastric type. On the basis of the histologic features, intestinal types were further classified as tubular type or tubulovillous type, and the gastric type was classified as foveolar type or pyloric-gland type. The clinicopathological features of these 4 types were examined.

Duodenal epithelial tumors were commonly found in elderly men and often arose in the proximal duodenum. Most tumors were intestinal type, whereas only about 10% were gastric type. The most frequent intestinal-type tumors were tubular-type lesions that grossly appeared as the flat-elevated type. The tubulovillous, foveolar, and pyloric-gland types were all large and characterized by prominent elevation. Gastric-type tumors tended to have higher atypia. Among the gastric types, the pyloric-gland type often had ectopic fundic glands, suggesting that these were special tumors with differentiation to fundic glands.

Urogenital research

1. Urological pathology in general: Differences in the clinicopathological and molecular biological features of prostate cancer on the basis of its site of origin were investigated. This study included 211 patients with prostate cancer who underwent total prostatectomy

at our hospital. The cancers were classified as anterior/posterior cancer and transition zone/peripheral zone cancer; and factors, such as cancer detection rates on needle biopsy and ERG expression on immunostaining, were compared. ERG expression of was significantly lower in anterior/transition-zone cancers than in posterior/peripheral-zone cancers. 2. Evaluation of overexpression of human epidermal growth factor receptor 2 in metastatic urothelial carcinomas: Immunohistochemical testing was performed to investigate whether human epidermal growth factor receptor 2 (HER2) was overexpressed in 116 patients with urothelial carcinoma. The immunohistochemistry score was 3 in 16.4% of cases. On the basis of the site, the score was 3 in 17.9% of urinary bladder cancers, 21.4% of ureteral cancers, and 5.6% of renal pelvic cancers. Overexpression of HER2 in urothelial carcinoma was seen more often in patients with lymph node metastases. Primary lesion and metastatic lesion scores were generally in agreement.

Female reproductive tract research

1. Histopathology in twins with complete mole and similar placentas: Many cases were diagnosed as partial moles, but some were complete moles with mosaicism. None had persistent trophoblastic disease.

2. Atypical polypoid adenomyomas of the endometrium: Clinicopathologic features of atypical polypoid adenomyomas in 71 patients, including our own cases and cases presented at conferences, were examined. The pathologic diagnosis on the basis of endometrial curettage specimens was difficult, and overdiagnosis as endometrioid adenocarcinoma was not uncommon. The clinical presentations were more diverse than previously reported and included cases of invasion into the myometrium and cases of lesions within adenomyosis. Complication by endometrioid adenocarcinoma was seen in 15 patients, but outcomes were good and no patients died. Persistent lesions were seen in all patients who underwent hysterectomy with repeat endometrial curettage or hormonal therapy. Hysterectomy is indicated for treatment, except for patients who strongly wish to preserve their fertility.

Respiratory tract research

1. Prominin 1: We investigated whether the protein prominin 1 was expressed and the significance of prominin 1 expression in 134 patients with surgically resected lung adenocarcinoma and 71 patients with squamous cell carcinoma (SCC). Prominin 1 was expressed in 64 (48%) patients with lung adenocarcinomas, suggesting a weak association between prominin 1 and the development of lung adenocarcinoma. However, prominin 1 was constitutively expressed by normal epithelial cells. This finding suggests that prominin 1 is important in maintaining the structure and function of normal bronchial mucosal epithelial cells. However, prominin 1 was expressed in only 9 (13%) patients with SCC. Therefore, prominin 1 is not a functional protein in cancer stem cells in the development of SCC.

2. The possibility of the microtubule associated tumor suppressor 1 (MTSU1) gene (*MTUS1*), located on the short arm of chromosome 8, as a novel candidate tumor suppressor gene was examined with microsatellite analysis. This subjects included 34 patients with earlier-stage hepatocellular carcinoma (HCC) and 22 patients (64 lesions; 22 pri-

mary lesions, 42 metastatic lesions) with advanced HCC and distant metastases. We investigated the expression of MTUS1 protein in the malignant transformation of hepatocytes using an anti-MTUS1 protein-specific antibody that we developed. The protein MTUS1 disappeared with malignant transformation in 75% of patients with HCC.

Soft tissue tumor research

1. Clinicopathologic features were examined in 8 patients with composite hemangioendotheliomas. The most common combination was retiform hemangioendothelioma and epithelioid hemangioendothelioma. Angiosarcoma was seen in some cases, but as in other patients, the outcomes were good.

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Department of Virology

Kazihiro Kondo, 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.

Research Activities

Reduction of adverse effects by a mushroom product, active hexose-correlated compound, in patients with advanced cancer during chemotherapy: The significance of the levels of HHV-6 DNA in saliva as a surrogate biomarker during chemotherapy

Chemotherapy improves the outcome of cancer treatment, but patients are sometimes forced to discontinue chemotherapy or drop out of a clinical trial because of adverse effects, such as gastrointestinal disturbances and suppression of bone marrow function. The objective of this study was to evaluate the safety and effectiveness of a mushroom product, active hexose correlated compound (AHCC), on chemotherapy-induced adverse effects and quality of life (QOL) in patients with cancer. Twenty-four patients with cancer received the first cycle of chemotherapy without AHCC and then received the second cycle with AHCC. During chemotherapy, we evaluated adverse effects and QOL weekly via a blood test, European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) questionnaire, and DNA levels of HHV-6 in saliva. The DNA levels of HHV-6 were significantly increased after chemotherapy. Interestingly, administration of AHCC significantly decreased the level of HHV-6 in saliva during chemotherapy and improved hematotoxicity and hepatotoxicity, as well as QOL scores in the EORTC QLQ-C30 questionnaire. These findings suggest that salivary HHV-6 levels are a good biomarker of QOL in patients during chemotherapy and that AHCC has a beneficial effect on chemotherapy-associated adverse effects and QOL in patients with cancer undergoing chemotherapy.

Effects of nutritional supplementation on fatigue, and autonomic and immune dysfunction in patients with end-stage renal disease: A randomized, double-blind, placebo-controlled, multicenter trial

Background: Fatigue is a predictor of cardiovascular events in patients with end-stage renal disease (ESRD) undergoing hemodialysis. We hypothesized that multinutritional support would improve QOL, symptoms of fatigue, and potential quantitative measures, including endocrine, immune and autonomic functions, in patients with ESRD undergo-

ing hemodialysis.

Methods: A total of 202 patients undergoing hemodialysis were randomly assigned to receive active treatment (containing vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12, folic acid, vitamin C, carnitine, coenzyme Q10, naïve galacto-oligosaccharide, and zinc) or a placebo after each dialysis session for 12 weeks. The patients and attending physicians were blinded to the treatment, and 172 patients (86 in each group) completed the study. Fatigue was evaluated via fatigue questionnaire at 0, 4, and 12 weeks. To assess the reactivation of HHV-6 and HHV-7, the number of viral DNA copies in saliva was determined with the polymerase chain reaction at weeks 0 and 12. Autonomic function was determined via measurement of beat-to-beat variation with acceleration plethysmography.

Results: The groups did not differ significantly in clinical characteristics, changes in fatigue, QOL score, endocrine functions, or laboratory data. Several measures of heart rate variability significantly increased after nutritional treatment compared with those in patients receiving placebo. Consumption of a nutritional drink for 12 weeks significantly suppressed HHV-7 DNA copy numbers. Similarly, HHV-6 DNA copy numbers tended to be decreased by treatment but without reaching statistical significance.

Conclusions: Nutritional supplementation may modulate immune and autonomic dysfunction in patients with ESRD undergoing hemodialysis.

HCMV latency-associated protein open reading frame 152 induces calcium influx and inhibits gene expression in nerve cells

The virus known as HCMV is the most common cause of congenital virus infection. Congenital HCMV infection causes birth defects and development abnormalities, including sensorineural hearing loss, microcephaly, intracranial calcification, and intrauterine growth retardation. However, the pathogenic mechanism remains unclear. We have previously found that the HCMV latency-associated protein open reading frame (ORF) 152 can interact with calcium modulating cyclophilin ligand (CAML), a cellular protein that regulates the intracellular calcium concentration, and significantly enhance intracellular calcium concentration in granulocyte-macrophage progenitor cells, which are sites of latent HCMV infection. Because calcium has tremendous effects on neural activities, we investigated the effect of ORF152 on neural abnormalities.

First, we examined whether HCMV latency-associated protein ORF152 is expressed in nerve cell lines. Next, we introduced ORF152 stably into the U373, A172, and SVGp12 cell lines and measured intracellular calcium concentration. Previously CAML has been reported to be required for efficient recycling of epidermal growth factor receptor (EGFR). Therefore, we determined the expression level of EGFR in these stable cell lines.

We found that ORF152 could be expressed around the nucleus in HCMV-infected nerve cell lines. Moreover, ORF152 induced intracellular calcium concentration and, when stably expressed, inhibited EGFR expression in nerve cell lines.

These results demonstrate that the HCMV latency-associated protein ORF152 induces intracellular calcium concentration and inhibits EGFR expression in nerve cell lines. It has been reported that EGFR knock-out mice have defects in cortical neurogenesis.

Therefore, ORF152 is suggested to have effects on nervous system and is a key factor of neuropathogenesis during congenital HCMV infection.

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

Yoshimitsu Mizunoe, Professor Tadayuki Iwase, Assistant Professor Kenichi Okuda, Assistant Professor Akiko Tajima, Assistant Professor Shinya Sugimoto, Assistant Professor

General Summary

Research projects of our department have focused on: (1) a novel strategy for biofilm inhibition with small molecules targeting molecular chaperone, (2) thioflavin T as a fluorescence probe for monitoring RNA metabolism, (3) analysis of staphylococcal biofilm structuring and dispersal, (4) biofilm formation with *Propionibacterium acnes* isolated from pacemakers, (5) high-throughput screening of antibiofilm compounds, (6) the role of gut microbes on the host nitrogen cycle, and (7) analysis of the viable but nonculturable state.

Research Activities

Novel strategy for biofilm inhibition with small molecules targeting molecular chaperone Biofilms are complex communities of microorganisms that attach to surfaces and are embedded in a self-produced extracellular matrix. Because these cells acquire increased tolerance against antimicrobial agents and host immune systems, biofilm-associated infectious diseases tend to become chronic. We show here that the molecular chaperone DnaK is important for biofilm formation and that chemical inhibition of DnaK cellular functions is effective in preventing biofilm development. Genetic, microbial, and microscopic analyses revealed that deletion of the dnaK gene (chaperone Hsp70, with co-chaperone DnaJ) markedly reduced the production of the extracellular functional amyloid curli, which contributes to the robustness of *Escherichia coli* biofilms. We tested the ability of the DnaK inhibitors myricetin, telmisartan, pancuronium bromide, and zafirlukast to prevent biofilm formation of E. coli. Only myricetin, a flavonol widely distributed in plants, inhibited biofilm formation in a concentration-dependent manner; however, it did not affect growth. Transmission electron microscopy demonstrated that myricetin inhibited the production of curli. These findings provide insights into the significance of DnaK in curli-dependent biofilm formation and indicate that DnaK is an ideal target for antibiofilm drugs.

Thioflavin T as a fluorescence probe for monitoring RNA metabolism

The intrinsically stochastic dynamics of messenger RNA (mRNA) metabolism have important consequences on gene regulation and nongenetic cell-to-cell variability; however, no generally applicable methods exist for studying such stochastic processes quantitatively. Here, we describe the use of the amyloid-binding probe thioflavin T for monitoring RNA metabolism in vitro and in vivo. Thioflavin T fluoresced more strongly in complex with bacterial total RNA than with genomic DNA. Thioflavin T bound purine oligoribonucleotides preferentially over pyrimidine oligoribonucleotides and oligodeoxyribonucleotides. Cellular analyses, in combination with genetic approaches and the transcription-inhibitor rifampicin treatment, demonstrated that thioflavin T stained mainly mRNA in actively dividing *E. coli* cells. Thioflavin T also facilitated mRNA metabolism profiling at the single-cell level in diverse bacteria. Furthermore, thioflavin T can also be used to visualize transitions between nonpersister and persister cell states, a phenomenon of isogenic subpopulations of antibiotic-sensitive bacteria that acquire tolerance to multiple antibiotics due to stochastically induced dormant states. Collectively, these results suggest that probing mRNA dynamics with thioflavin T is a broadly applicable approach ranging from the molecular level to the single-cell level.

Analysis of staphylococcal biofilm structuring and dispersal

Staphylococcus aureus colonizes prosthetic implants as biofilm, which is multiple layers of bacteria. Inside the biofilm, bacteria are embedded in self-produced matrixes, such as extracellular DNA (eDNA), proteins, and polysaccharide, which elevate levels of resistance to antibiotics and host defenses. Dispersal of biofilm can result in spread to secondary sites and worsening of the infection.

In the staphylococcal biofilm development, bacteria formed biofilm within 8 hours in the growth medium brain-heart infusion with or without glucose; however, biofilm was dispersed in the brain-heart infusion without glucose after 24 hours. Analysis of the extracellular matrix of biofilm showed that this dispersal correlated with degradation of eDNA or extracellular RNA (eRNA) in the matrix. The addition of DNase I had no effect on biofilm dispersal, but RNase A caused biofilm disassembly and inhibited biofilm formation. This finding suggests that eRNA is important for biofilm structure. The culture supernatant from dispersed biofilm caused biofilm disassembly, which suggested biofilm was dispersed by a self-produced secretion factor. It has been reported that *S. aureus* produces thermonuclease (nuclease A, 16.8 kDa; nuclease B, 18.8 kDa). A fraction of 10 to 50 kDa was applied to a cation exchange column, and the active fraction showed DNase activity in DNA zymography. These results suggest the importance of eRNA in biofilm formation and dispersal. Analysis of biofilm disassembly mechanism is in progress.

Biofilm formation by P. acnes isolated from pacemaker

The bacterium *P. acnes* is a facultative anaerobic Gram-positive commensal of the human skin, mouth, conjunctiva, and large intestine. It is usually responsible for late chronic infections and rarely causes acute infections related to medical devices. In this study, the colonization of bacteria on the surfaces of explanted cardiac devices (pacemaker generators) that show no signs of infection was consecutively analyzed. As a result of culture tests with agar plates followed by 16S ribosomal RNA gene sequencings, *P. acnes* was isolated from 7 of 31 devices. To examine whether certain lineages associate with characteristics of the *P. acnes* isolates, sequence typing of *P. acnes* were categorized into 4 different sequence types (STs), including ST2 (2 isolates), ST4 (1 isolate), ST53 (1 isolate), ST6 (2 isolates), and novel ST (1 isolate). As a result of a biofilm formation assay using polystyrene plate, biofilm formations of all *P. acnes* isolates were enhanced by addition

of glucose in growth media. Sensitivity testing of biofilms against proteinase K, DNase I, and dipersin B, which target potential biofilm matrix components, suggested that eDNA is important for the formation and stability of biofilm. This work was supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT)-supported Program for the Strategic Research Foundation at Private Universities, 2012-2016.

High-throughput screening of antibiofilm compounds

One of the potential strategies for preventing and treating biofilm-associated infections is to use small molecules that inhibit biofilm development. We are now pushing ahead high-throughput screening to identify the compounds effective against bacterial biofilm development in collaboration with the University of Tokyo, which has a chemically diverse small-molecule library. Before now, screening has been run with 59,600 compounds, and 2 compounds — ABC-JK1 and ABC-JK2 — that show strong antibiofilm activity against staphylococci, including methicillin-resistant *S. aureus*, were obtained. Both ABC-JK1 and ABC-JK2 affected the extracellular polysaccharide synthesis and cell wall synthesis of staphylococci. Molecular mechanisms of action for ABC-JK1 and ABC-JK1 are now under investigation with a multi-omics approach that uses transcriptomics, proteomics, and metabolomics. This work was supported by the MEXT-supported Program for the Strategic Research Foundation at Private Universities, 2012-2016.

Role of gut microbe on host nitrogen cycle

Nitrogen, like oxygen, hydrogen, and carbon, is an important element for the growth, maintenance, and survival of organisms. Nitrogen itself is abundantly present on Earth; however, it predominantly exists in the air as molecular nitrogen, which is inactive and cannot be utilized by organisms. Compared with the amount of the bioavailable forms of other elements, that of 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.

Publications

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Department of Tropical Medicine

Hirotaka Kanuka, Professor

Kenji Ishiwata, Associate Professor

General Summary

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

Research Activities

Mutual interaction by the mixed infection of 2 species of gastrointestinal nematode Human gastrointestinal (GI) nematodes usually make chronic and mixed infections. Host immunity to the parasites evokes a response of helper T type 2 cells (Th2) followed by changing the environment of the intestinal tract dynamically, but the responses fail to drive them away from the gut. Murine experiments have shown that the worm expulsion depends largely on interleukin 13/signal transducer and activator of transcription (STAT) 6 signaling. Nippostrongylus brasiliensis, a murine GI nematode, penetrates subcutaneously, migrates the lung, reaches the small intestine by day 3 postinfection, and is expelled interleukin 13/STAT6-dependently by day 10 postinfection. Orally infected Heligmosomoides polygyrus, another murine GI nematode, grows in the submucosa of the small intestine, returns into the lumen on day 8 postinfection, and also develops a Th2 response. It survives in the tract for more than 2 months. Here, to investigate mutual interaction of 2 different kinds of GI nematode, H. polygyrus infection, regarded as a human infection model, was combined with N. brasiliensis infection. We examined whether N. brasiliensis could extend its parasitic period when N. brasiliensis was infected at the time H. polygyrus established well in the gut, and we also examined whether H. polygyrus was expelled with N. brasiliensis when H. polygyrus appeared back to the lumen at the time N. brasiliensis was expelled. Results suggested that (1) the parasite infected ahead expended its parasitic period with following infection of the other species, (2) consecutively infected parasite underwent influence of the host immunity led by the preceding infection parasite, and (3) the egg production ability of female worms was easily affected by the host immunity apart from worm expulsion. Although single infection experiments have raised an idea that an expulsion mechanism is different depending on the parasite species, this experiment system reflecting a phenomenon of the natural world will provide a fresh perspective in host immune response-mediated mutual interaction between the parasites living together.

Ejection of pathogen-invaded cell maintains midgut wall of malaria vector mosquito The midgut of disease vectors contributes as a primary and most important physical barrier against pathogens. The overview of antipathogen responses has been unclear, and 2 conflicting models of mosquito midgut cells and *Plasmodium* ookinete invasion have remain unsettled. One model proposed the existence of distinct midgut epithelial cells without microvilli, called Ross cells, into which ookinete preferentially invades; the other proposed midgut wall consists of indistinguishable epithelial cells that change morphologically similar to Ross cells when invaded by ookinete. To reveal the conformation of midgut cells before and after invasion by ookinete, the midgut of mosquitoes (Anopheles stephensi) infected with rodent malaria parasite (*Plasmoidum berghei*) was analyzed precisely with confocal microscope for its function as a barrier. The infected midgut wall contained extruding cells, which had no microvilli, with its adjacent cells of both sides contacting in the basal part. Ookinetes were often observed inside or adjacent to extruding cells, indicating that invaded cells were extruded while ookinete migrated to neighboring cells. Meanwhile, the midgut from mosquitoes that had ingested noninfected blood showed a midgut wall with indistinguishable microvillar columnar cells. Our observations, supporting the latter model, demonstrated that mosquitoes positively eliminate parasites by ejecting parasite-invaded cells, whereas parasites that have invaded midgut cells migrate from cell to cell to escape from mosquito's elimination mechanisms. This competition between mosquitoes and parasites may explain the natural balance of vector tolerance and the number of parasites.

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 (SFTS) virus is a newly identified *Phlebovirus* genus in the Bunyaviridae family causing acute hemorrhagic fevers in parts of East Asia, including China, Korea, and Japan. The SFTS virus has been detected and isolated from diverse species of ticks in the endemic areas. We collected ticks from 15 point localities (over an area of 10 km²), on the island of Kyushu, 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 (with a white flannel cloth of 170×70 cm). The SFTS virus-specific RNA was detected with the reverse-transcription polymerase chain reaction (RT-PCR) in complementary DNA generated from the RNA of individual ticks. The SFTS virus-specific RNA was detected in 4 species: Haemaphysalis formosensis, Haemaphysalis longicornis, Haemaphysalis flava, and Haemaphysalis hystricis. The SFTS virus-specific RNA was not detected with RT-PCR in any field-collected Ambryomma testudinarium. Of the ticks collected in April, most (n = 794) were nymphs (SFTS virus 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, SFTS virus-specific RNA was also detected in larvae. Larvae may become infected with the SFTS virus via vertical transmission.

Genetic dissection of intermediate host and tapeworm interaction

The dwarf tapeworm, *Hymenolepis nana*, which belongs to the order *Cyclophyllidea*, is the most common cestode of humans. Its intermediate hosts are 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 examine the interaction between the tapeworm and the 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 (dsRNA) into late instar larvae. We performed RNAi screening targeting several gene transcripts of the Toll and the immune deficiency pathways, which are major signaling pathways of the humoral immune response in insects. Reduction of Toll pathway function, which was induced by RNAi-mediated silencing of myeloid differentiation primary response 88 (MvD88), Dif1, and Dif2, in addition to Janus kinase/STAT and c-Jun N-terminal kinase components, increased the burden of cysticercoids. On the other hand, RNAi-mediated knockdown of immune deficiency pathway components, Death related ced-3/Nedd2-like caspase (Dredd) and immune deficiency (imd), had no significant effect on the cysticercoid load. Our findings suggest a pivotal role of specific pathways, such as the Toll signaling pathway, in regulating resistance to tapeworm infection.

The role of gut in Plasmodium-transmitting vector mosquito

Vector-borne diseases rely upon organisms, named vectors, such as mosquitoes, ticks, and sandflies, that have an active role in the transmission of a pathogen from one host to the other. A critical stage in pathogen transmission occurs in the vector midgut, when the pathogens ingested with blood first makes contact with the gut epithelial surface. To understand the response mechanisms within the midgut environment, including those influenced by resident microbiota against pathogens, we focus on both midgut bacteria species and the vector-pathogen interaction that confers diversity to the vector's competency for pathogen transmission. *Serratia marcescens* isolated from either laboratory-reared mosquitoes or wild populations in Burkina Faso shows great phenotypic variation in its cellular and structural features. Importantly, this variation is directly correlated with its ability to inhibit *Plasmodium* development within the mosquito midgut. Furthermore, this anti-*Plasmodium* function conferred by *S. marcescens* requires increased expression of the flagellum biosynthetic pathway that is modulated by the motility master regulatory operon, flhDC. These findings point to new strategies for controlling malaria through genetic manipulation of midgut bacteria within the mosquito.

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Department of Public Health and Environmental Medicine

Hiroyuki Yanagisawa, Professor Shingo Yogosawa, Assistant Professor Machi Suka, Associate Professor

General Summary

Our major research projects in the 2014 academic year focused on: (1) effects of Zn-deficient and Zn-excess ingestion on gene expression of inflammatory cytokines and chemokines in spleen macrophages in Spague Dawley rats; (2) potential mechanisms responsible for tubulointerstitial nephropathy induced by fluoride in Spague Dawley rats with unilateral ureteral obstruction; (3) transgenerational effects of maternal arsenic exposure; (4) molecular approaches toward cancer chemoprevention with food factors; (5) the decompression stress in the hyperbaric work; (6) effects of electric and magnetic fields; (7) relationships among health literacy, health information access, health behavior, and health status; (8) determination of reading comprehension of health checkup reports; (9) 8-week open-label trial of polaprezinc, a zinc complex, on treatment of pressure ulcer healing; (10) time-to-effect relationships between systolic blood pressure and the risks of nephropathy and retinopathy in patients with type 2 diabetes; and (11) association between glycosylated hemoglobin (HbA1c) variability and mortality in patients with type 2 diabetes.

Research Activities

Experimental Medicine

1. Gene expression of inflammatory cytokines and chemokines in spleen macrophages derived from rats fed Zn-deficient or Zn-excess diets

A deficiency of Zn causes growth retardation and dysfunction of the immune and reproductive systems. The present study was designed to examine the expression of inflammatory cytokines and chemokines in spleen macrophages from rats fed a Zn-deficient diet, a standard diet, or a Zn-excess diet for 6 weeks. Gene expression of *111b*, *Tnf*, *Cc12* and *Cc13b* was significantly higher in rats fed a Zn-deficient diet than in rats fed a standard diet or a Zn-excess diet.

2. 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 seriously affected by fluoride. In this study, we used the tubulointerstitial fibrosis model (unilateral ureteral obstruction). We examined whether fluoride deteriorates the tubulointerstitial nephropathy seen in rats with unilateral ureteral obstruction.

3. Effects of maternal arsenic exposure on later generations of C3H mice

Our recent study showed that maternal arsenic exposure of C3H mice increases hepatic

tumors in male offspring and grandchildren at 74 weeks of age. In this study, we focused on other target organs. Gene expression analysis of the lungs and kidneys with real-time reverse transcriptase-polymerase chain reaction method showed differences between control and arsenic groups in male offspring and grandchildren.

4. 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 and apoptosis.

5. A study of decompression stress in hyperbaric work

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

6. Biological effect of electric and magnetic fields

To evaluate the biological effects of electric and magnetic fields, various mutagenicity assays were employed with various exposure conditions. As a result, no mutagenic effect was observed except after long-term exposure to a strong static magnetic field greater than 2 T. At 20 kHz, we found no effect on differentiation of mouse embryonic stem cells to myocardial cells.

Epidemiology, evidence-based medicine, investigation, and medical informatics

1. Relationship between health literacy, health information access, health behavior, and health status

A questionnaire survey was performed among health examinees at 6 healthcare facilities. We examined the relationship between health literacy, health information access, health behavior, and health status.

2. Reading comprehension of health checkup reports

A Web-based survey was performed among a sample drawn from a research panel. We determine the reading comprehension of health checkup reports in the context of health literacy.

3. Oral treatment of pressure ulcers with polaprezinc (zinc L-carnosine complex): 8-week open-label trial

We performed an open-label trial of polaprezinc with maximum 8-week treatment for chronic pressure ulcers. The results suggested that polaprezinc is effective and well tolerated and could be a candidate for the oral treatment of pressure ulcers.

4. Time-to-effect relationships between systolic blood pressure and the risks of nephropathy and retinopathy in patients with type 2 diabetes

The time-to-effect relationship with systolic blood pressure (SBP) differed for the development of nephropathy and retinopathy. The long-term effect was clear for nephropathy and was borderline for retinopathy, whereas the short-term effect was stronger and evident for both. Continuous lowering of SBP is necessary to prevent nephropathy, whereas control of SBP during the preceding 5 years seems to be important to prevent retinopathy. 5. Association between HbA1c variability and mortality in patients with type 2 diabetes We investigated the association between the variability of HbA1c and mortality due to all causes — cancer and noncancer, including cardiovascular disease — in patients with type 2 diabetes. The variability of HbA1c is a predictor of all-cause mortality, especially non-cancer mortality including cardiovascular disease independent of the mean HbA1c level. In contrast, mean HbA1c, but not HbA1c variability, might predict cancer mortality.

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Department of Forensic Medicine

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

General Summary

Our main research projects in 2014 have mainly focused on forensic pathology, DNA analysis, and forensic toxicology as has happened in the past. Much of the research was based on forensic practice. The details of our research are described below.

Research Activities

Forensic pathology

1. An experimental study of ligature marks

The macroscopic findings of ligature marks are mainly affected by 3 factors: the characteristics of the ligature, the severity of external force upon the ligature, and the length of time the neck was pressed by the ligature. However, few quantitative or experimental studies have examined which of the latter 2 factors contributes more significantly to determine the appearance of ligature marks. We experimentally made artificial ligature marks by hanging on legs of rats, both intravitally and posthumously. Macroscopic examination and statistical analysis revealed that the appearance of ligature marks is not affected by whether the marks are formed intravitally or postmortemly, but equally affected by the severity of force and the duration of force application. Histological examination, with hematoxylin and eosin stain and Elastica Masson-Goldner (EMG) stain, revealed that we could distinguish the compressed area from the other area but could not distinguish intracitally or postmortemly. Immunostaining methods for fibronectine, α 1-antichymotrypsin, and CD31 could not distinguish either the area or the time.

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, and 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. Studies of a simple DNA extraction method from various types of forensic samples: Application to chewing gum

We have investigated an extraction method from chewing gum to obtain a sufficient amount of DNA for analyzing mitochondrial DNA and short tandem repeats. In particular, we have focused on preventing contamination with, for example, foreign DNA and polymerase chain reaction inhibitors.

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 meconin

We detected meconin in an autopsy case. Meconin is an organic compound included in opium which can be detected in the urine after opium inhalation. Therefore, detecting meconin from biological specimens is important in opiate diagnosis. Qualitative and quantitative methods of analyzing meconin with gas chromatography/mass spectrometry were examined.

3. Examination of a method for analyzing 4'-methyl- α -pyrrolidinohexiophenone and citalopram

We detected metabolites of 4'-methyl- α -pyrrolidinohexiophenone, which is a designer drug, and citalopram, which is an antidepressant drug of the selective serotonin reuptake inhibitor class, in autopsy cases. Qualitative and quantitative methods of analyzing these drugs were examined with gas chromatography/mass spectrometry.

Radiocarbon analysis

1. Establishment of age estimation

We studied estimating the date of birth from carbon-14 isolated from a tooth. The duration of enamel formation is generally shorter than that of dentin formation. Therefore, to estimate the date of birth with greater precision, enamel should be used instead of dentin. On the other hand, to establish whether the age range was before or after the peak atmospheric concentration of carbon-14 in 1963, when above-ground nuclear bomb tests were banned, the use of dentin is better because dentin formation occurs over a longer time. We have investigated a method of specifying the age range from a tooth by combining the measured carbon-14 level of enamel and dentin.

Publications

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Department of Internal Medicine Division of Gastroenterology and Hepatology

Hisao Tajiri, Professor Yoshio Aizawa, Professor Shigeo Koido, Associate Professor Atsushi Hokari, Associate Professor Kazuhiko Koike, Assistant Professor Yoshinari Miyagawa, Assistant Professor Yasushi Onoda, Assistant Professor Shinichiro Uetake, Assistant Professor Akiyoshi Kinoshita, Assistant Professor Toshifumi Okusa, Professor Hirokazu Nishino, Professor Tomohisa Ishikawa, Associate Professor Mika Matsuoka, Assistant Professor Satoshi Suto, Assistant Professor Seiji Arihiro, Assistant Professor Hiroshi Abe, Assistant Professor Masayuki Saruta, Assistant Professor Kan Uchiyama, Assistant Professor

Research Activities

Alimentary Tract

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 score, and Matts' grade (histologic activity) for PEG-MUM were each higher than those for C-reactive protein. Compared with the C-reactive protein level, the PGE-MUM level demonstrated greater sensitivity for reflecting ulcerative colitis activity, especially in cases of histologic inflammation, and thus seems to be a better evaluator of mucosal healing.

2. Efficacy of infliximab in preventing restenosis after the endoscopic balloon dilatation therapy for Crohn's disease

The safety and efficacy of infliximab for preventing restenosis after endoscopic balloon dilatation were evaluated in 14 patients with Crohn's disease. Ten patients had no restenosies when infliximab was administered. Our results suggest that infliximab is useful for preventing restenosis after the endoscopic balloon dilatation therapy in patiens with Crohn's disease.

3. Photodymamic diagnosis of colitis-associated cancer or dysplasia withautofluorescent endoscopy following 5-animolevulinic acid sensitization

On the basis of previous animal experiments, we performed autofluorescent endoscopy after oral administration of 5-animolevulinic acid in 11 patients with ulcerative colitis. Of 21 lesions with the characteristic fluorescence signals, 14 received pathological diagnoses of colitis-associated cancer or dysplasia. The nethod of diagnosis showed good positive and negative predictive values and a sensitivity and specificity. Autofluorescent endoscopy following 5-animolevulinic acid sensitization would offer a useful method for diagnosing colitis-associated cancerous lesions.

4. Development of optical molecular imaging for gastrointestinal cancer and imageguided phototherapy

We have developed photoimmunotherapy, a type of molecular target-specific phototherapy that uses monoclonal antibodies conjugated to the near-infrared phthalocyanine dye. We have established a molecular target-specific phototherapy that uses imaging-guided and fluorescence molecular imaging methods in a mouse model of human gastric cancer.

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

We examined clinical features of preoperatively diagnosed early gastric cancer treated with endoscopic submucosal dissection, particularly those with synchronous and metachronous multiple tumors.

6. Nutritional therapy for inflammatory bowel diseases

We demonstrated that understanding and using the omega-3 diet method, which emphasizes the intake of omega-3 polyunsaturated fatty acid, improves the efficacy of maintaining the remission of inflammatory bowel diseases.

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

We found that the 94C>A mutation of the inosine triphosphate pyrophosphatase gene (ITPA) is involved in the onset of thiopurine-induced leukopenia in Japanese patients with inflammatory bowel disease.

Pancreas

We demonstrated that a higher frequency of memory-phenotype Wilm's tumor protein 1 (WT1)-specific cytotoxic T lymphocytes could be a useful prognostic marker for a good response to the combination therapy with gemcitabine and WT1-pulsed dendritic cell vaccines in patients with advanced pancreatic cancer.

Liver

1. Pathogenesis of minimal hepatic encephalopathy

We found that psychometric testing was a useful method for the early diagnosis of minimal hepatic encephalopathy.

2. Nutritional imbalance of patients with liver cirrhosis

W e examined the nutritional status of patients with liver cirrhosis. The nutritional background was analyzed with a food frequency questionnaire based on food groups. We could easily evaluate the relation between nutritional imbalance and morbidity.

3. Nutritional evaluation in nonalcoholic fatty liver disease

The pathogenesis of nonalcoholic fatty liver disease resembles metabolic syndrome. We evaluated nutritional conditions in detail in nonalcoholic fatty liver disease. On the basis of the results, we are attempting to develop a new nutritional intervention for nonalcoholic fatty liver disease.

4. Clinical characteristics of primary biliary cirrhosis and autoimmune antibodies

Autoimmune antibodies against mitochondria, nuclei, or nuclear pore glycoprotein 210 (gp210) were examined in patients with primary biliary cirrhosis. The pattern of autoimmune antibodies was analyzed in association with the clinical course, outcome, and histological findings.

5. A useful prognostic factor in cases of hepatocellular carcinoma

The Glasgow Prognostic Score system, based on inflammation criteria and including only serum C-reactive protein and albumin, showed a correlation with prognosis in cases of

hepatocellular carcinoma.

6. Response to antiviral nucleic acid analogs in chronic hepatitis B virus infection

Resistant viral mutations are a urgent remedial problem in cases of chronic hepatitis B virus infection treated with antiviral nucleic acid analogues. We repeatedly analyzed viral gene sequences and followed up the treatment response rate. We consider the possibility of a new concurrent therapy for chronic hepatitis B virus infection. We found that the differences in clinical characteristics depend on the viral genotype. Cases of infection with the genotype A virus were more likely to be severe, to be prolonged, and to be treated with an antiviral nucleic acid analogue.

7. Immunological analyses in a mouse model of autoimmune hepatitis

Intrahepatic natural killer T-cell and several cytokine profiles were examined in a mouse model of autoimmune hepatitis.

8. Antiviral therapy against chronic hepatitis C virus infection

We investigated the efficacy of telaprevir-based combination therapy for genotype 1b chronic hepatitis C virus (HCV) infection in poor-responders to interferon (interleukin 28B minor). We found that the response to treatment is associated with serum HCV dynamics and the level of 25-hydroxyvitamin D3.

9. Abnormal lipoprotein kinetics in chronic HCV infection

We found that reduced serum levels of apolipoproteins CII and CIII are a common feature of chronic HCV infection regardless of HCV genotype although the kinetics of apolipoproteins E and A1 depends on the HCV genotype.

10. A biomarker for nonalcoholic steatohepatitis

We demonstrated that the serum level of cytokeratin 18 fragments is a useful marker for diagnosis and evaluation of activity for nonalcoholic steatohepatitis.

11. Clinical features of superelderly patients with hepatocellular carcinoma

We investigated clinical features and prognosis of superelderly patients with inoperable hepatocellular carcinoma.

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Department of Internal Medicine Division of Neurology

Yasuyuki Iguchi, Professor Masahiko Suzuki, Associate Professor Hiroshi Yaguchi, Assistant Professor Yu Kono, Assistant Professor Shusaku Omoto, Assistant Professor Hisayoshi Oka, Professor Kazutaka Matsui, Assistant Professor Chizuko Toyoda, Assistant Professor Renpei Sengoku, Assistant Professor

General Summary

Our clinical research in 2014 was performed in the following areas: 1) neurodegenerative disease, 2) cerebrovascular disease, and 3) autoimmune disease.

Research Activities

Clinical researches

Neurodegenerative disease

1. Randomized, double-blind, placebo-controlled trial of vitamin D supplement to prevent deterioration in Parkinson's disease

Parkinson's disease (PD) is a debilitating movement disorder, and its incidence is growing because of the aging of societies worldwide. In our previous study, higher levels of 25-hydroxyvitamin D and the vitamin D receptor *Fok*I CC genotype were associated with milder PD. We evaluated whether vitamin D3 supplementation inhibits PD progression subgrouped by vitamin D receptor genotypes without triggering hypercalcemia. A total of 114 patients with PD were randomly assigned to receive vitamin D3 supplements (n = 56; 1200 IU/day) or placebo (n = 58) for 12 months in a double-blind setting. In conclusion, vitamin D3 supplementation may prevent worsening of symptoms in patients with *Fok*I TT or CT genotypes without triggering hypercalcemia.

2. Electroencephalogram in PD

In patients with PD, slow waves are typically demonstrated with electroencephalography (EEG), but considerably fast waves also appear with them. On the hypothesis that released cortex affected by the restraint from basal ganglia to the cerebral cortex generates the surplus activity as fast waves on the EEG. We measured PD with EEG to examine the local existence of fast waves and their correlation with slow waves.

3. Evaluation of autonomic symptoms in de novo PD (with self-assessment questionnaires)

We collected the Survey of Autonomic Symptoms and the Japanese Sialorrhea Clinical Scale for Parkinson's Disease and evaluated the Unified Parkinson's Disease Rating Scale, orthostatic hypotention, and [¹²³I]*meta*-iodobenzylguanidine (MIBG) myocardinal scintigraphy in patients with de novo PD. The existence of orthostatic hypotension was not correlated with the complaints of lightheadedness. Before being treated, patients with PD had cold feet and constipation. The complaints of leaking of urine were correlated with orthostatic hypotension in patients with de novo PD.

4. The usefulness of dopamine transporter single-photon emission computed tomography for reassessment of parkinsonian disorders

Dopamine transporter (DAT) single-photon emission computed tomography (SPECT) has been available since 2014 in Japan. It is the method used to detect presynaptic dopamine neuronal dysfunction, which is a hallmark of neurodegenerative parkinsonian disorders. We verified the usefulness of DAT SPECT for reassessing parkinsonian disorders that were diagnosed with conventional neuroimaging methods. We believe DAT SPECT is useful for excluding drug-induced parkinsonism or vascular parkinsonism from neurodegenerative parkinsonian disorders.

5. The quantitative motion analysis using portable gait rhythmogram after cerebrospinal fluid drainage in idiopathic normal pressure hydrocephalus

We aimed to reveal the quantitative gait differences between idiopathic normal pressure hydrocephalus, PD, and normal controls using portable gait rhythmogram (PGR). The PGR revealed narrower and more monotonous step lengths in patients with idiopathic normal pressure hydrocephalus than in patients with PD or healthy control subjects. In addition, PGR detected gait improvements following cerebrospinal fluid (CSF) drainage in patients with a CSF drainage response. Thus, PGR can easily be used to analyze long durational gait in daily life and can usefully provide additional quantitative data on former measurements of CSF drainage.

6. Clinical characteristics of supine hypertension in de novo PD

Older age, akinetic-rigid motor subtype, and preexistent hypertension are independent risk factors for supine hypertension. Supine hypertension may be associated with peripheral sympathetic nervous denervation that is milder than that associated with orthostatic hypotension. As for global cognitive decline, supine hypertension is a far riskier comorbidity of early-stage PD than is orthostatic hypotension.

7. Blood pressure fluctuation and clinical features in de novo PD

In patients with PD, daily fluctuation of blood pressure has been previously reported. Our aim was to investigate the relationship between blood pressure fluctuation and clinical characteristics in de novo PD. We found a correlation between the nocturnal reduction of systolic blood pressure and cardiac MIBG uptake. These results may help reveal features of the autonomic symptoms of PD.

8. Dysphagia and vocal cord palsy in multiple system atrophy

In cases of advanced multiple system atrophy, symptoms that are not rare include dysphagia and vocal cord palsy. Vocal cord palsy is a life-threatening risk at the time of percutaneous endoscopic gastrostomy (PEG). We estimated dysphagia and vocal cord palsy with laryngoscope in cases of multiple system atrophy. In many cases vocal cord palsy was presented at the time of PEG. In such cases, PEG was performed safely with noninvasive positive pressure ventilation therapy.

9. The relevance of hyposmia and MIBG scintigraphy in multiple system atrophy

Findings of denervative olfactory failure in MIBG myocardial scintigraphy are useful for discriminating PD and multiple system atrophy (MSA). We examined the relationship between olfactory failure and MIBG myocardial scintigraphy in patients with MSA at our hospital.

Of patients with MSA, 17% showed findings of denervation on MIBG myocardial scin-

tigraphy and 11% showed olfactory failure. However, no patients showed both findings. Olfactory dysfunction and MIBG myocardial scintigraphy were not related in patients with MSA.

Cerebrovascular disease

1. Sonothrombolysis for hyperacute stroke with low-frequency and fluctuating-frequency transducer

A new 500 ± 200 -kHz transducer promoted higher penetration through the skull without any distractive effects, such as extra enhancement of sonographic power. This transducer enhanced thrombolytic effects in comparison with the power of a standard 500-kHz transducer.

2. Evaluation of right-to-left shunt with a novel probe attached to the cervix

The purpose of this study was to compare the diagnostic accuracy of a novel pasteable soft ultrasound probe (PSUP) with that of transesophageal echocardiography (TEE) for right-to-left shunt. Subjects were patients with ischemic stroke and transient ischemic attack who underwent TEE. First, we performed TEE with a standard protocol for detecting a right-to-left shunt. Then, monitoring with a PSUP was performed at a common carotid artery with a similar preparation and procedure to those of TEE. For the diagnosis of TEE, the PSUP had a sensitivity of 83%, a specificity of 93%, and accuracy of 91%. The PSUP may be useful for patients with an insufficient temporal bone window.

3. Clinical characteristics of vertebrobasilar dolicoectasia in patients with cerebral infarction

Vertebrobasilar dolicoectasia (VBD) is a rare condition characterized by significant expansion and elongation of the vetebrobasilar arteries. However, the association of VBD with cerebral infarction has not yet been studied. Our aim was to investigate the clinical characteristics of VBD in patients with cerebral infarction. We enrolled 103 patients; of these patients, 9 (6%) had VBD. Distinct characteristics of VBD in patients with cerebral infarction and the absence of cardiac embolism. These characteristics may play an important role in the mechanism of VBD in patients with cerebral infarction.

4. The relationship between small-vessel disease and intracranial vessel resistance

The neurological deterioration associated with the enlargement of a lesion is often seen in patients with small-vessel disease. The aim of this study was to clarify the relationship between the size of lesions in small-vessel disease and the evaluated factors by the transcranial Doppler.

5. Clinical characteristics of recurrence of cardiogenic cerebral embolisms in the acute phase

When treatment with oral anticoagulants should be started for secondary prevention in patients with acute ischemic stroke is unclear. Our aim was to investigate the clinical characteristics of cerebral embolic stroke and its recurrence in the acute phase. Of the 72 patients examined only 40% had received oral anticoagulants before admission. Recurrence in the acute phase was observed in 13% of all patients, and symptomatic recurrence was observed in 10%.

6. Grade of cortical vessel signs on susceptibility-weighted imaging predicts outcomes in

acute stroke patients

Cortical vessel signs (CVSs) on susceptibility-weighted magnetic resonance imaging (SWI) are frequently observed in patients with an acute ischemic stroke. However, the clinical implications of this sign have not yet been clearly defined. We assumed the hypothesis that the grade of CVSs on SWI predicts outcomes in patients with acute stroke. As with grade of CVSs, the proportional rate of unfavorable patients gradually increased (P < 0.01). The grade of CVSs on SWI can predict outcomes in patients with acute stroke.

Autoimmune disease

1. Single photon emission computed tomography findings after vaccination against human papillomavirus in Japan

We demonstrated relative hypoperfusion areas in 3-dimensional stereotactic surface projections with single event effect (SEE) analysis were most prominent in the cingulate gyrus in patients after human papillomavirus (HPV) vaccinations. Our study strongly suggested that various clinical symptoms in patients with HPV vaccination associated with neuroimmunopathic syndrome were caused by central nervous system impairment after HPV 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 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 with proteinuria to dialysis and kidney transplantation. Therefore, our research groups are investigating diverse subjects and aim to eventually find new therapeutic strategies and mechanisms of disease progression, which may help decrease the number of patients with end-stage renal diseases.

Research Activities

Studies on immunoglobulin A nephropathy

We demonstrated that steroid pulse therapy with tonsillectomy had an independent effect on the disappearance of proteinuria (Nephrol Dial Transplant 2014). We also analyzed the prognostic factor that affects the recurrence of immunoglobulin A nephropathy after steroid pulse therapy (under revision).

Studies on low glomerular density in glomerular diseases

Our studies showed that low glomerular density was strongly associated with the prognosis of various glomerular diseases (Clin Kidney J 2014, Hypertens Res 2015, Am J Hypertens 2015). Collaborative research about the estimation of nephron numbers in Japanese patients is in progress.

Studies of hypertension and renal damage

We analyzed renal histopathological findings in relation to ambulatory blood pressure values. Only the severity of interstitial damage exhibited a significant association with an increased value of ambulatory blood pressure (Hypertens Res 2015).

Studies of chronic kidney disease-mineral and bone disease

In basic research, we evaluated the effects of chronic kidney disease on the transcription factor glial cells missing 2 (Gcm2), which is indispensable to parathyroid gland and epigenetic variations.

Also, we analyze functions of glial cells missing 1 (*Gcm1*), which is a homolog of *Gcm2*. In a clinical study we clarified that ferric citrate hydrate, a novel iron-based phosphate binder, decreased concentrations of fibroblast growth factor 23. Because novel iron-based

phosphate binders increase serum ferritin levels (CJASN 2014), we evaluate that there is no association between mortality and anemia related parameters included ferritin, hemoglobin, and transferrin saturation among patients undergoing dialysis with the registry date of the Japanese Society for Dialysis Therapy (NDT 2014).

Studies of peritoneal dialysis

We confirmed the availability of combined therapy with peritoneal dialysis and hemodialysis using outcomes of combined therapy in a cohort of more than 100 patients (Blood Purification 2014). Moreover, we found that survival outcome of combined therapy was not worse than that of peritoneal dialysis or hemodialysis (Blood Purification 2014). We are evaluating peritoneal injury using laparoscopy.

Study of renal transplantation

1. We investigated the significance of caveolin 1 immunoreactivities in peritubular capillaries in patients who have undergone the transplantation of kidney from living related donors. 2. We analyzed the effect of medullary ray injury early after kidney transplantation on the graft survival. 3. We examined the difference between ABO-compatible and ABO-incompatible kidney transplantation regarding cytomegalovirus infection.

Renal protective effects of azilsartan in adenine-induced renal failure model rats

We examined the mechanism of the renal protective effects of azilsartan in a rat model of renal failure. Daily urinary sodium excretion was decreased in the nonmedication group, and azilsartan suppressed the decreasing sodium excretion. Sympathetic nerve activity was elevated by azilsartan. Blood pressure was not elevated in this experimental model; therefore, because azilsartan greatly suppressed blood pressure, it did not suppress sympathetic nervous system, as previously reported.

Central blood pressure and activity of the renin-angiotensin-aldosterone system

We examined the relationship between central blood pressure (CBP) and the renin-angiotensin-aldosterone system in patients with primary aldosteronism and essential hypertension. The gap between central blood pressure (CBP) and brachial systolic blood pressure (SBP) increased with the plasma aldosterone concentration in essential hypertension. In primary aldosteronism, the CBP-SBP gap was significantly higher than that in essential hypertension. This study suggests that, even if SBP is well controlled, the kinetics of CBP indicate a different tendency from SBP as the renin-angiotensin-aldosterone system increases and might increase the risk of cardiovascular events.

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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 autoimmune disease.

1. Evaluation and analysis of synovial blood flow signals of patients with rheumatoid arthritis on power Doppler ultrasonography

We have previously demonstrated that the serum level of vascular endothelial growth factor is significantly correlated with disease activity in patients with rheumatoid arthritis (RA). We evaluated RA disease activity before and after administration of abatacept or tocilizumab and examined whether this administration affects serum levels of angiogenesis-related factors and the synovial blood flow signals in patient's joints measured with power Doppler ultrasonography. Our data have demonstrated that both tocilizumab and abatacept decreased the disease activity but that tocilizumab decreased the vascular endothelial growth factor level and synovial blood flow signals more quickly than did abatacept.

2. 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. This year, we have examined whether fasciitis is detectable with power Doppler ultrasonography in patients with dermatomyositis.

3. Analysis of telomerase activity in peripheral blood mononuclear cells of patients with autoimmune disease

Telomerase activation is observed in healthy cells, including normal lymphocytes. An increase in telomerase activity is associated with the activation of lymphocytes. Much attention has been paid to the role of telomerase in immunocytes. This year we measured telomerase activity in peripheral blood mononuclear cells obtained from patients with autoimmune diseases, especially RA.

4. Citrullination of chemokines in RA

Citrullination, catalysed by peptidylarginine deiminase, 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.

5. Bombina variegata peptide 8/prokineticin 2: a novel arthritis-inducible chemokine The chemokine Bombina variegata peptide 8 (Bv8)/prokineticin 2 is related to angiogenesis, circadian rhythm, and the lowering of the pain threshold. We have previously shown that Bv8 is highly expressed in synovial tissues in mice with collagen-induced arthritis. However, the mechanism of Bv8 regarding the onset of arthritis remains unknown. We examined whether Bv8 can recruit polymorphonuclear leukocytes (PMNs) or monocytes *in vitro* and induce inflammatory arthritis *in vivo*. Our data showed that Bv8 recruited PMNs *in vitro* and induced PMN-driven inflammatory arthritis *in vivo*. These results suggest that Bv8 contributes to the pathogenesis of RA. Targeting Bv8 may provide a new therapeutic strategy to treat inflammatory arthritis.

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Department of Internal Medicine Division of Cardiology

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

General Summary

We have 6 research groups for covering the broad field of cardiology. We perform studies from both clinical and basic standpoints in each research group. We aspire to achieve a greater understanding of the pathogenesis of cardiovascular diseases and to establish or improve clinical diagnostic methods and therapies.

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 (IHD), into a database. Using this precise database, we have been performing a study comparing risk factors, clinical outcomes, and other data. In addition, we have participated in nationwide clinical studies, such as J-LESSON (Japan Unprotected Left Main Coronary Artery Disease Percutaneous Coronary Intervention Strategy On New Generation Stents), PROPEL (A Prospective Multicenter Post-Approval Study to Evaluate the Long-Term Efficacy and Safety of the Resolute Integrity in the Japanese All-Comers Patients with Coronary Artery Disease), and NIPPON (Nobori Dual Antiplatelet Therapy as Appropriate Duration). In 2014, we reported the following interesting clinical findings. We found that the malondialdehyde-modified (MDA) low-density lipoprotein (LDL) level was affected by multiple factors, such as smoking status, LDL-C, and the male sex. Furthermore, statin therapy might have a beneficial effect on the reduction of the MDA-LDL level. Also, we reported about B-type natriuretic peptide (BNP) in patients with IHD. Although plasma BNP levels are increased in patients with heart failure and acute myocardial infarction, we reported that plasma BNP levels were lower in stable patients with IHD than in stable patients without IHD. Perhaps the low reactivity of BNP is causally associated with IHD.

Arrhythmia Research Group

In our arrhythmia team, we have been focusing on the management of atrial fibrillation (AF) among various types of arrhythmias. Each year we perform ablation for more than 400 patients, including 300 patients with AF. We have reported new findings or new methods of catheter ablation at international conferences or in published journals, such as

factors associated with the recurrence of AF after ablation, better methods for minimizing the recurrence of AF after ablation, and the association of sleep apnea with the outcome of AF ablation. In 2014, we started a new ablation method with cryoballoons for curing paroxysmal AF.

Heart Failure Research Group

We have been examining clinical data related to the plasma levels of BNP, which is a sensitive marker of heart failure. Body mass index is a significant factor that reduces the plasma BNP level. This effect is significantly increased in patients with a high body mass index, even among those with a worsening severity of heart failure. Also, we reported about aldosterone in a collaboration study; the immunolocalization of calcium channels in nonpathological adrenals and idiopathic hyperaldosteronism were detected in the zona glomerulosa, with a predominance of CaV3.2 channels in aldosterone-producing adenoma. These findings suggest that different types of calcium channel can be involved in calcium-related aldosterone biosynthesis.

Imaging Research Group

Multidetector (row) computed tomography (MDCT) has become a reliable method for detecting coronary arterial organic stenosis. We have been studying the possibility that a change in coronary arterial tonus can also be detected with repeated MDCT. In 2014, we confirmed for the first time that coronary arteries can fluctuate substantially and that these changes can be documented with MDCT. Changes in coronary arterial tonus should therefore be considered when reading MDCT.

Molecular Biology Research Group

Although the utilization of fatty acids is the predominant metabolic pathway in the normal adult heart, glucose becomes an important preferential substrate for metabolism and ATP generation under specific pathological conditions, such as ischemia. Therefore, the acceleration of glycolysis and glucose utilization in the ischemic myocardium may be cardioprotective. We found that the sodium-dependent glucose co-transporter (SGLT) 1, a major glucose transporter in the heart, is highly expressed in human hearts. Using the Langendorff heart perfusion system, we demonstrated that cardiac SGLT1 provides an important protective mechanism against ischemia-reperfusion injury by replenishing ATP stores in ischemic cardiac tissues by enhancing the availability of glucose. The present findings provide new insights into the significant role of SGLTs in optimizing cardiac energy metabolism, at least during the acute phase of ischemia-reperfusion injury.

Cardiac Physiology Research Group

We have investigated cardiac physiology and pathophysiology, especially cardiac Ca^{2+} handling and adrenergic signaling related to excitation-contraction coupling. We have also reported the role of thrombin on cardiac disease, leading to a next experiment using a mouse model of dilated cardiomyopathy. The activation of protease activated receptor (PAR) 1 is known to lead to the expression of α -smooth muscle actin and to contribute to atrial pathological fibrosis. We examined which types of PAR contribute to the profibrotic

activity of cultured neonatal rat atrial fibroblasts and demonstrated the profibrotic activity of PAR-1 but not of PAR-2, -3, or -4.

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Department of Internal Medicine Division of Diabetes, Metabolism and Endocrinology

Kazunori Utsunomiya, Professor Kuninobu Yokota, Professor Masami Nemoto, Associate Professor Rimei Nishimura, Associate Professor Hironari Sano, Assistant Professor Kei Fujimoto, Assistant Professor Daiji Kawanami, Assistant Professor Katsuyoshi Tojo, Professor Yutaka Mori, Professor Tamotsu Yokota, Associate Professor Shuichi Kato, Assistant Professor Takanori Ebisawa, Assistant Professor Masaya Sakamoto, 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 Takaki Shimada, Associate Professor Hidekazu Masuoka, Assistant Professor Shingo Yano, Assistant Professor Yoji Ogasawara, Assistant Professor Takeshi Saito, Assistant Professor Noriko Usui, Professor 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 throughout 2014.

Research Activities

Leukemias

Many patients with previously untreated hematological disorders have been referred to our department. The disorders in 2014 included acute myeloid leukemia (AML), 22 cases; acute lymphoblastic leukemia (ALL), 5 cases; chronic myeloid leukemia (CML), 8 case; and myelodysplastic syndrome (MDS), 13 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 the clinical research and treatment of AML, ALL, and CML. The JALSG protocol studies performed in 2014 were as follows: AML-209-GS, AML209-KIT, JALSG-ALL-CS-12, JALSG-CS-11, JALSG AML209-FLT3-SCT Study (AML209-FLT3-SCT), JALSG CML212, a Phase II JALSG APL212 study, APL212G, JALSG MDS212 study, JALSG MDS212 Study (MDS212), JALSG Ph(–)B-ALL213, JALSG Burkitt-ALL213, and JALSG T-ALL213-O. JALSG Ph+ALL213, JALSG-STIM213. 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/I).

Lymphomas

In 2014 we registered 88 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 studies JCOG0406 (newly diagnosed mantle cell lymphoma: phase II) and JCOG0601 (newly diagnosed low-risk advanced diffuse large B-cell lymphoma: phase II/III) were pivotal protocol studies beginning in 2010. A randomized phase II study in patients with high-risk diffuse large B-cell lymphoma has also

been started (biweekly rituximab-cyclophosphamide, doxorubicin, vincristine, and prednisone [bi-R-CHOP] ± 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 12 patients with newly diagnosed multiple myeloma in 2014. 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. In-house protocols are also being prepared.

Hematopoietic stem cell transplantation

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

Solid tumors

Many patients with solid cancers have been referred to our department from related divisions or departments from both inside and outside our hospital. Several of our studies seeking improved therapeutic outcomes are in progress throughout our university hospital with related divisions or departments. The combination of fluorouracil (5-FU), epirubicin, and cyclophosphamide (FEC100) with or without taxotere therapy is an adjuvant therapy for patients with breast cancer treated with curative surgery. FEC100 followed by taxotere is a preoperative combination chemotherapy for patients with locally advanced breast cancer. Adriamycin and taxotere followed by taxotere and trastuzumab is a first-line chemotherapy for patients with advanced, metastatic breast cancer. Since late 2008 we have been investigating a combined-modality therapy of radiation and chemotherapy with docetaxel, cisplatin, and 24 hours' continuous infusion of 5-FU (DCF regimen) for patients with locally advanced esophageal cancer. The study has been completed, and an improved protocol was launched 2 years ago. A novel drug-development study with an orally decaying formulation of S-1 has been performed in patients with advanced gastric cancer. A multicenter cooperative randomized phase II study was started in 2011 to compare S-1 + cisplatin, S-1 + leucovorin, and S-1 + leucovorin + oxaliplatin for patients with advanced and recurrent gastric cancer. Because trastuzumab is also active in patients with human epidermal growth factor receptor 2-overexpressing gastric cancer, we treat such patients with capecitabine + cisplatin (XP) + trastuzumab. Our first-line chemotherapies for patients with advanced colorectal cancer are folinic acid, fluorouracil, and oxaliplatin (FOLFOX) and folinic acid, 5-FU, and irinotecan (FOLFIRI). Since antibodies against vascular endothelial growth factor and against epidermal growth factor receptor became available in 2007 and 2008, respectively, combination therapies of these antibodies and FOLFOX or FOLFILI have also been performed. Salvage therapies using regorafenib or TAS102 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. The structural differences between M protein produced by myeloma cells and that from monoclonal gammmopathy of undetermined significance have been examined, and the function of ATP-binding cassette transporters in cancer chemotherapy has also been studied in collaboration with Keio University's Department of Pharmacy. Transfer of the *MDR1* gene into hematopoietic stem cells is a method of potentially conferring chemoprotection in cancer chemotherapy. Basic research using CD34-postive cells allows us to try such a strategy. The growth and differentiation of CD34-postive cells into which the *MDR1* gene has been transferred has been investigated *in vitro* in collaboration with Keio University's Department of Pharmacy. The results have recently been published, and further research is 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 Akira Kojima, Professor Jun Araya, Associate Professor Hiromichi Hara, Assistant Professor

General Summary

We performed 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 lead to novel treatments. We completed clinical research concerning COPD in collaboration with the Department of Cardiology and the Department of Diabetes, Metabolism, and Endocrinology, and a manuscript has been submitted. Basic research focusing on the molecular mechanisms of lung injury, fibrosis, and COPD is in progress. We have specifically investigated the roles of apoptosis, cellular senescence, and autophagy in the pathogenesis of various lung diseases.

Research Activities

Cellular senescence and autophagy in COPD

COPD is caused by the noxious effects of tobacco smoke, which lead to airway epithelial cell injury and the induction of phenotypic changes, such as squamous metaplasia and cellular senescence, which are assumed to be part of the adaptive response to toxic components, such as reactive oxygen species (ROS). The acceleration of cell senescence induced by cigarette smoke has been widely implicated in the pathogenesis of COPD. The accumulation of damaged proteins and organelles are typical manifestations of cell senescence, indicating the involvement of autophagy, a bulk degradation pathway for cellular components, in the regulation of cell senescence in COPD. We found that treatment of human bronchial epithelial cells (HBECs) with cigarette smoke extract (CSE) transiently induced activation of autophagy, which was associated with accelerated cellular senescence and concomitant accumulations of p62 and ubiquitinated proteins. Autophagy induction in response to CSE was significantly decreased in HBECs from patients with COPD, and levels of both p62 and ubiquitinated protein were increased in lung homogenates from these patients, suggesting the involvement of insufficient p62-mediated selective autophagic clearance of ubiquitinated proteins in accelerated cellular senescence in the pathogenesis of COPD (Fujii S, Oncoimmunology 1: 630-641, 2012).

Mitochondria are dynamic organelles that are essential for cellular metabolic functions and continuously change their shape through fission and fusion. The proper regulation of mitochondrial dynamics is crucial for the maintenance of functional mitochondria and, hence, disruption of dynamics induces excessive production of ROS, resulting in apoptosis and cellular senescence. Accelerated cellular senescence is implicated in the pathogen-
esis of COPD. Accordingly, we investigated the involvement of mitochondrial dynamics in CSE-induced cellular senescence in HBECs. Treatment with CSE induced both mitochondrial fragmentation and mitochondrial oxidative stress, which were responsible for the acceleration of cellular senescence in HBECs. Both mitochondrial fragmentation and mitochondrial oxidative stress induced by CSE treatment were inhibited in the presence of N-acetylcysteine or Mito-TEMPO. Mitochondrial fragmentation induced by knockdown of fusion proteins also increased mitochondrial ROS production and the percentage of senescent cells. Mitochondrial fragmentation induced by CSE is involved in cellular senescence through the mechanism of mitochondrial ROS production. Hence, disruption of mitochondrial dynamics may be a part of the pathogenic sequence by which COPD develops (Hara H, *et al.*: *Am J Physiol Lung Cell Mol Physiol* 305: L737-746, 2013).

We are investigating the role of sirtuin 6 (SIRT6), which is a member of the sirtuin family of proteins. Sirtuins play important roles in antiaging or anticellular senescence. We investigated the role of SIRT6 in the pathogenesis of COPD. Knockdown of SIRT6 by small interfering RNA induced cellular senescence in human bronchial epithelial cells, and overexpression of SIRT6 attenuated cellular senescence induced by CSE. We found that SIRT6 inhibited the insulin-like growth factor-akt-mechanistic target of rapamycin pathway, which is the major cellular senescence pathway. The mechanistic target of rapamycin inhibits autophagy activation. Therefore, SIRT6 activates autophagy flux and attenuates cellular senescence (Takasaka N, et al. J Immunol 2014).

Cellular senescence and autophagy in idiopathic pulmonary fibrosis

Aberrant re-epithelialization with bronchial epithelial cells is a prominent pathologic finding in idiopathic pulmonary fibrosis (IPF) and is implicated in abnormal epithelialmesenchymal interactions. Recent studies have shown that senescence is a risk factor for the development of IPF. Among the sirtuin family of class III histone deacetylases, SIRT6 has been shown to antagonize senescence. We examined epithelial senescence as a representative phenotypic alteration in conjunction with SIRT6 expression in IPF. We have produced evidence that IPF lungs show enhanced senescence with a concomitant increase in SIRT6 expression in epithelial cells, including aberrantly re-epithelialized bronchial cells. Transforming growth factor (TGF)- β induces senescence by increasing p21 expression and also induces SIRT6 expression, and artificial overexpression of SIRT6 efficiently inhibits TGF- β -induced senescence via proteasomal degradation of p21 in HBECs. Secretion of interleukin β 1 from TGF- β -induced senescent HBECs is responsible for myofibroblast differentiation in fibroblasts. 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).

Accelerated epithelial cell senescence accompanied by excessive myofibroblast proliferation has been implicated in the pathogenesis of IPF. Autophagy plays an important regulatory role in cellular senescence and differentiation. To determine if insufficient autophagy is involved in the pathogenesis of IPF, the regulatory role of autophagy in cell senescence and myofibroblast differentiation was tested with in-vitro models. We also examined the autophagy status using immunohistochemial evaluation of microtubule-associated protein light chain 3 (LC3), beclin 1, p62, and ubiquitin in the lung. Autophagy has been shown to prevent cellular senescence caused by tunicamycin-induced endoplasmic reticulum stress in HBECs. Conversely, autophagy inhibition was sufficient to induce myofibroblast differentiation in lung fibroblasts. We also demonstrated that metaplastic epithelial cells and fibroblasts in fibroblastic foci expressed both ubiquitinated proteins and p62 in IPF. Cellular senescence, as measured by p21 expression and senescence-associated β -galactosidase staining, was observed in metaplastic epithelial cells covering fibrosing lesions. Type II alveolar epithelial cells in relatively normal areas of IPF exhibited ubiquitin staining; however, a concomitant increase in LC3, indicating autophagy activation, may explain why p21 expression was not observed in these cells. These findings suggest that insufficient autophagy is a potent underlying pathology of both accelerated cellular senescence and myofibroblast differentiation in a cell-type-specific manner and is a promising clue for understanding the molecular mechanisms of IPF (Araya J, Am J Physiol Lung Cell Mol Physiol 304: L56-69, 2013).

We are also investigating the role of prostaglandin E_2 (PGE₂),which is an important molecule associating with fibrogenesis. We measured the concentration of metabolites of PGE₂ in urine from patients with IPF. The concentration of PGE₂ metabolites was significantly greater in urine from patients with IPF than from control subjects. The manuscript is being prepared.

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

The microorganisms most commonly associated with acute exacerbation of bronchial asthma (AEBA) are respiratory viruses, such as rhinovirus, and atypical bacteria, such as Mycoplasma pneumonia. Causative organisms of AEBA in pediatric populations have been well documented but are rarely reported in adults. Recently, multiplex polymerase chain reaction (PCR) has been used to effectively detect both respiratory bacteria and viruses. To evaluate etiologies in adult AEBA, a rapid, reliable process based on real-time (RT)-PCR for respiratory samples was used. We prospectively enrolled adult patients with AEBA who met our criteria: 20 years or older, within 7 days of onset, and informed consent. Nasopharyngeal swabs and sputum samples were collected from all patients, and comprehensive RT- PCR was used to detect 6 bacteria and 11 respiratory viruses. Of the 36 patients who satisfied our criteria, 25 (69.4%) had microorganisms, either bacteria or viruses or both, which were detected with PCR. The diagnosis was viral infection in 7 patients (19.4%), bacterial infection in 11 patients (30.6%), atypical bacterial infection in 3 patients (8.3%), and viral/bacterial co-infection in 4 patients (11.1%). The remaining 11 patients (30.6 %) had unknown pathogens. The most common microorganisms were Haemophilus influenzae, M. pneumonia, and rhinovirus. Our results suggest that RT- PCR analysis of nasopharyngeal swabs and sputum samples is helpful for determining the cause of AEBA in adults. Results of the detection of *M. pneumonia* and rhinovirus were as expected; however, the detection of *H. influenzae* was unexpected. On the basis of these results, we analyzed the association between microorganisms and AEBA. These results were presented at the European Respiratory Society meeting and are being prepared for journal submission.

Publications

Fujita Y, Yoshioka Y¹, Ito S, Araya J, Kuwano K, Ochiya T¹ (¹Natl Cancer Ctr Hosp). Intercellular communication by extracellular vesicles and their microRNAs in asthma. *Clin Ther.* 2014; **36**: 873-81.

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Fujiwara **T**^{1,2,3}, *Kawai* **A**², *Nezu* **Y**¹, *Fujita* **Y**¹, *Kosaka* **N**¹, *Ozaki* **T**³, *Ochiya* **T** (¹*Natl Cancer Ctr Res Inst,* ²*Natl Cancer Hosp,* ³*Okayama Univ)*. Circulating microRNAs in sarcoma: potential biomarkers for diagnosis and targets for therapy. *Chemotherapy.* 2014; **3**: 123.

Reviews and Books

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Department of Internal Medicine Division of General Medicine

Iwao Ohno, Professor Joji Otsuki, Associate Professor Nobuyuki Furutani, Associate Professor Hideo Okonogi, Assistant Professor Yasuhiko Miura, Assistant Professor Hiroshi Yoshida, Professor Masami Nemoto, Associate Professor Jun Hiramoto, Associate Professor Takanori Ebisawa, 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, complaints, whether the patient had consulted other physicians, the primary diagnosis, examinations, and care. This year, the most frequent reasons for consultation were abdominal pain, cough, and pyrexia. The most frequent initial diagnoses were 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 primary healthcare, including community healthcare. Thus, we are now planning postgraduate and life-long training programs for physicians to acquire skills for the general practice required in the community.

Division of General Medicine, The Jikei University Katsushika Medical Center

We presented several case reports associated with endocrine diseases: pituitary disease, adrenal disease, thyroid disease, and electrolyte abnormality.

We examined the factors that affected the serum procalcitonin level. Although procalcitonin is used as an inflammatory marker of bacterial sepsis, we examined the association of procalcitonin with thyroid hormones and glucose metabolism. A total of 169 patients in our hospital (24 to 96 years old; 90 man and 79 woman) were divided into 3 groups on the basis of procalcitonin concentration (≥ 2 ng/ml, 33 patients; 0.5-2 ng/ml, 108 patients, and < 0.5 ng/ml, 28 patients). A procalcitonin concentration was associated with increases in C-reactive protein, white blood cells, and neutrophils and a decrease in lymphocytes. We found not association of procalcitonin with thyroidal function, but procalcitonin had strong correlations with blood glucose and HbA1c. *Klebsiella pneumoniae, Staphylococcus pneumoniae, Escherichia coli*, and *Hemolytic streptococcus* were isolated from blood cultures of patients with procalcitonin ≥ 2 ng/ml. *Campylobacter* and *Candida* were isolated from the blood cultures of patients with procalcitonin < 0.5 ng/ml.

Division of General Medicine, The Jikei University Daisan Hospital

1. Study of polymyalgia rheumatic

We found that morning stiffness is present in only 30% of patients with polymyalgia rheumatica. A high level of C-reactive protein was found in all patients, and an elevation of matrix metalloproteinase 3 was found in 80% of patients. Mild cases of polymyalgia rheumatica can be treated into remission with 10 mg of prednisolone. We can cure about 50% of cases in this way, but other cases require continuous treatment.

2. Study of hyponatremia in elderly patients

Hyponatremia is a common electrolyte disorder in elderly patients. The syndrome of inappropriate secretion of antidiuretic hormone caused by minor stress due to inflammation is the main cause of hyponatremia in elderly patients.

3. Study of sepsis

Changes in the white blood cell count and levels of procalcitonin and C-reactive protein have limitations as markers for the early diagnosis of sepsis. We began to measure the new marker presepsin.

Division of General Medicine, The Jikei University Kashiwa Hospital

Our research in The Jikei University Kashiwa Hospital consists of 4 parts. The first is to develop interprofessional work in Kashiwa. We conducted an open seminar related to general medicine. The second part is to develop educational tasks for teaching medical students and junior physicians. We developed the education system using e-portfolio. Education program concerning medical-professionalism and medical-ethics are running. The third part is to develop the core competency of the Hospitalists in Japan, especially in university hospitals. The fourth part is to establish a system for a Hospital Ethics Committee and Clinical Ethics Consultation in The Jikei University Kashiwa Hospital.

Department of Psychiatry

Kazuhiko Nakayama, Professor Kei Nakamura, Professor Hironari Sue, Professor Wataru Yamadera, Associate Professor Kazuya Ono, Associate Professor Ayumu Tateno, Assistant Professor Koji Nakamura, Assistant Professor Minako Koga, Assistant Professor Hiroshi Itoh, Professor Hisatsugu Miyata, Professor Kazutaka Nukariya, Associate Professor Motohiro Ozone, Associate Professor Rieko Shioji, Assistant Professor Tatsuhiko Itoh, Assistant Professor Norifumi Tsuno, 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 attentions tended to decrease. In the field of psychotherapy, we attempted to develop a prototype of dialectical behavior therapy for Japanese patients, diary therapy to maintain self-esteem in patients with autistic disorders, and cognitive functions for patients with developmental disorders.

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 have been continuously promoting comparative studies between Morita therapy and "the third generation" of cognitive-behavioral therapies. Recently, we started practical research towards the application of Morita therapy to adolescent patients. 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 novel anti-craving agent (the final 3 subjects were performed in collaboration with the NTT Communication Science Laboratories and the Department of Psychology, Senshu University). In clinical research, we performed the following studies in humans: 1) the developmental and the psychological and social predictors of recovery in patients with schizophrenia study, 2) effects of antipsychotics on dopamine transporter binding using positron emission tomography, 3) regulation of the salience network with dopamine D2/3 receptors via antipsychotic agents, 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 compensatory neural mechanism of anosognosia in Alzheimer's disease (AD) and investigated the neural correlates of anosognosia in AD. We found that anosognosia in AD could be correlated with executive dysfunction and functional compensation through the semantic memory system. Another study revealed that the presence of subcortical white matter hyperintensities in patients with late-life somatoform disorders was a predictor of disease severity. Cognitive dysfunction appeared to play a role in the advancement of disease severity. 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 are performing a clinical study of the management of pregnancy in woman with epilepsy. We discussed changes in the serum concentrations of antiepileptic drugs during the pregnancy. Furthermore, we reported a case of epilepsy which was induced by a specific situation and showed a peculiar clinical course. 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 Ms. Yukiko Kurokawa to a clinical conference and studied "psychotherapy for the aged," and how to use it clinically. Furthermore, we trained graduate students of a clinical psychological course.

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Department of Pediatrics

Hiroyuki Ida, Professor Mitsuyoshi Urashima, Professor Toshio Katsunuma, Associate Professor Yoshihiro Saito, Associate Professor Hiroshi Kobayashi, Associate Professor Hiroshi Tachimoto, Assistant Professor Masahisa Kobayashi, Assistant Professor Toya Ohashi, Professor Ichiro Miyata, Associate Professor Yasuyuki Wada, 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 group is to supply practical benefits to patients and their families through basic and translational research and clinical study.

Inherited metabolic disease group

In bone marrow transplantation for murine models of mucopolysaccharidosis type II, we clarified the relation between donor chimerism and therapeutic efficacy, in collaboration with hematology and oncology group, and clarified the usefulness of anti-cKit antibody as a preconditioning. We developed an immune tolerance induction method through the oral administration of enzyme in enzyme-replacement therapy for Pome disease. Using cardiomyocytes differentiated from human Pome disease-induced pluripotent stem cells, we demonstrated the feasibility of gene therapy and found that the elevation of oxidized glutathione indicates the presence of oxidative stress. We had certain preclinical results, but we will attempt to apply these results to patients We performed genetic diagnosis with a comparative genomic hybridization array and exome sequencing in patients with congenital anomalies and intellectual disabilities.

Neurology group

We investigated the prognoses in 35 children with hypoxic encephalopathy at more than 1 year since onset. The sequelae were physical disabilities in 28 cases, mental disabilities in 30, epilepsy in 16, and higher brain dysfunction in 12. Continuous performance of the programs based on proper evaluation was a necessary in daily living.

We evaluated the efficacy of fosphenytoin for status epilepticus. Fosphenytoin was effective in 70% of 24 children. Transient hypotension in 1 patient was observed as an adverse effect. Administration of fosphenytoin (22.5 mg/kg) could not maintain the optimal phenytoin level after 15 hours, and maintenance therapy (7.5 mg/kg) could not maintain an optimal level.

Nephrology group

We identified possible risk factors for acute kidney injury following cardiac surgery. We found that postoperative acute kidney injury was associated with age < 1 year, cardiac surgery of the Risk Adjusted Classification for Congenital Heart Surgery grade ≥ 4 , and cardiopulmonary bypass time > 90 minutes.

Infectious diseases and Immunologic Disorders group

In this year, we conducted clinical trial of *ex vivo* autologous hematopoietic stem cell gene transfer as salvage therapy for patient with X-linked chronic granulomatous disease (CGD). We showed that gene therapy was able to provide life-saving clinical benefit to patients with CGD lacking a suitable donor.

We also studied human herpes virus (HHV) 6 reactivation in the central nerve system using astrocytoma cell line in collaboration with the Department of Virology. We demonstrated that interleukin 1 β and basic fibroblast growth factor are important factors for the propagation and reactivation of HHV-6 and are involved in the pathogenesis of HHV-6 encephalopathy.

Hematology and Oncology group

We performed mutational analyses with the comprehensive Cancer Panel to evaluate the molecular mechanism to develop malignancies associated with congenital anomaly syndrome. We reported on a Japanese boy with phosphoglycerate kinase (PGK) deficiency presenting as chronic hemolytic anemia. The PGK 1 gene (*PGK1*) sequencing showed a novel missense mutation: c. 1180A>G (PGK-Aoto) at exon 10.

Late sequelae, 20 years after onset, in adult survivors of solid tumors during childhood were reported. Even with complete remission of cancer, these survivors had multiple late sequelae or chronic health condition or both.

We studied the pain management and pediatric palliative care. Based on the result of a cross-sectional survey of Tokyo Children's Cancer Study Group, "pain management during bone marrow aspiration and biopsy in pediatric cancer patients" was published.

Cardiology group

The pediatric cardiology group is interested in both basic and clinical cardiology research to improve outcomes for children with congenital or secondary heart disease. Ongoing projects are as follows.

1. Right heart failure and peroxisomal proliferator-activated receptor gamma

2. Hepatic fibrosis and brain mitochondrial deformity in the status of heart failure

3. Right ventricular fibrosis in the status of pulmonary hypertension and pulmonary stenosis

4. Establishment of an aortopulmonary shunt with pulmonary artery banding rats in hypoxia

5. Urocoltin and angiopoietin evaluation in congenital heart disease

Our research on right heart failure and peroxisomal proliferator-activated receptor gamma received the Young Investigator Award at the annual meeting of the Association for European Paediatric Cardiology

Allergy group

The main subjects of our research are as follows: 1) the role of eosinophils, 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 been organized and performed the following multicenter clinical studies: the ORIMA study (Effect of ORal Immunotherapy in preschool children with Milk Allergy; Trial for the detection of prospective markers for the effectiveness) 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.

Endocrinology group

To clarify the mechanism of puberty, we analyzed gonadotropin-inhibitory hormone (GnIH)-knockout mice. The onset of puberty of GnIH-deficient mice was slightly earlier than that of wild-type mice. In male mice, body weight of GnIH-knockout models was significantly lighter than that of wild-type models at the onset of puberty. Further analysis is underway.

We investigated 28 boys with precocious puberty. High prevalence of organic diseases in male patients with precocious puberty was confirmed. We especially reported that necessity of paying attention to the progression of precocious puberty at a younger age. On the other hand, we identified 3 novel mutations of the solute carrier family 16, member 2 (thyroid hormone transporter) gene (*SLC16A2*) in 3 patients with suspected deficiency of monocarboxylic acid transporter 8 and found new clinical aspects.

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Department of Dermatology

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

General Summary

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

Research Activities

Psoriasis

Various systemic therapies, including oral cyclosporin microemulsion preconcentrate, methotrexate, etretinate, biologics and topical therapies such as vitamin D3, and corticosteroids, have been used, depending on disease severity and the degree to which quality of life (QOL) has been impaired in individual patients. Also phototherapy, including psoralen plus ultraviolet A, narrow-band ultraviolet B (UVB), and the 308-nm excimer lamp, have been administered in the skin-care clinic with considerable efficacy. We have evaluated patients' QOL reflecting social background and have developed a Japanese version of the Psoriasis Disability Index. We also developed a Japanese version of the Work Productivity and Activity Impairment questionnaire for psoriasis. We examined the incidence of metabolic syndromes as a comorbidity of 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 severe, intractable psoriasis. Clinical trials have been performed with new biologic agents, including antibodies against interleukin (IL)-17A, IL-17 receptor, IL-23p19, and new topical agents.

Atopic dermatitis

The pathogenesis of atopic dermatitis has been attributed to a complex interaction of environmental factors, host susceptibility genes, altered skin barrier function, and the immune system. Recently, psychosocial factors have been suggested to influence the exacerbation of atopic dermatitis. Therefore, we are now 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 have evaluated the patients' sleep quality using the Pittsburgh Sleep Quality Index and have found that its score was positively correlated with the scores of Severity Scoring of Atopic Dermatitis Index and Dermatology Life Quality Index, indicating that nocturnal itching and scratching behavior impair the sleep quality of patients with atopic dermatitis.

In basic clinical research, the levels of substance P, thymus and activation-regulated chemokine (TARC), and IL-31 related to pruritus in atopic dermatitis are being evaluated according to disease severity. Clinical trials of anti-IL-31 RA monoclonal antibody have been performed.

Malignant skin tumors

We have been studying clinical courses, postoperative outcomes, and genomic and expression changes in patients with malignant melanoma, extramammary Paget's disease, squamous cell carcinoma, basal cell carcinoma, cutaneous T-cell lymphomas, and a wide variety of sarcomas, including malignant peripheral nerve sheath tumor (MPNST). For the accurate diagnosis of pigmented tumors, we always perform dermatoscopic examinations and sentinel lymph-node biopsy, especially for patients with stage II or III melanoma. Now we are studying the clinical significance of sentinel lymph-node navigation surgery in extramammary Paget's disease. We are participating in collaborative clinical research for maintenance therapy using local injections of interferon β and in several nationwide epidemiological studies.

Neurofibromatosis

Because the number of registered patients in our clinic is the largest in Japan and because many patients with letters of introduction visit from all over 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%, although information concerning the epigenetic abnormality is limited. We have used the methylation-specific 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 and MPNSTs from patients. The findings of abnormal expression of several cancer-testis genes and the inactivation of tumor-suppressor genes indicate that disarranged methylation and demethylation are involved in the ontogenesis of MPNST.

Herpes virus infection

1. Herpes simplex virus

We treat patients with genital herpes and intractable oral/facial herpes. Rapid diagnostic procedures by means of immunohistochemical staining with monoclonal antibodies against herpes simplex virus (HSV)-1, HSV-2, and varicella-zoster virus (VZV) are performed in this clinic. We also perform enzyme-linked immunosorbent assays of antibodies against HSV glycoproteins G-1 and G-2 for patients with genital herpes to determine the type of HSV. After the diagnosis is confirmed, suppressive therapies (patient-initiated therapy and episodic therapy) with varaciclovir are started to improve the impaired QOL. 2. Herpes zoster and post-herpetic neuralgia

Initial treatments for herpes zoster and post-herpetic neuralgia (PHN) are performed in this clinic. Neurological complications are commonly associated with herpes zoster. PHN,

defined as pain present for 90 days after the onset of rash, is a major sequela of VZV infection and impairs QOL. To prevent PHN, we proactively use tricyclic antidepressants. Post-hoc analyses of a subgroup of patients has shown that amitriptyline in combination with acyclovir reduces the incidence of PHN. PHN is characterized by various types of pain and sensory symptoms, including ongoing pain, allodynia, and evoked or spontaneous intermittent lancinating pains. We prescribe pregabalin, tricyclic antidepressants, selective serotonin reuptake inhibitors, and opioid analgesics, such as Tramcet[®] (Grunethal Ltd., Stokenchurch, UK),which contains tramadol hydrochloride and acetoaminophen. Tramadol is a weak μ -opioid receptor agonist that induces serotonin release and inhibits the reuptake of noradrenaline. We use visual analogue scales and an objective measuring device (Pain Vision PS-2100, Nipro Co., Osaka) to evaluate the effect of treatment.

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 infection typing with PCR has regularly been performed for bowenoid papulosis and rare viral warts. Five-percent imiquimod cream is also available for the treatment of condyloma acuminatum.

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 because of its selective photothermolysis. Such treatment is covered by health insurance. Senile freckles are usually successfully treated with a single treatment, but because treatment is not covered by health insurance, it is performed at the patient's personal expense. 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 efficacy of a pulsed dye laser for treating hemangiomas and telangiectasia depends on the clinical type, location, patient age, and other factors. The pulsed dye laser was effective for treating hemangioma simplex on the face or neck of young adults. The size and redness of the strawberry mark can be reduced if treatment is started before the age of 6 months. The recently introduced V-beam laser is effective for intractable vascular lesions. We have been able to use the V-beam laser since 2011. Because the ultra-pulse CO_2 laser has higher energy and a shorter pulse width, it can vaporize at a fixed depth and can be used to quickly remove actinic keratosis, seborrheic keratosis, syringoma, and epidermal nevus.

Skin Care Clinic

Narrow-band UVB irradiation is performed for patients with psoriasis, alopecia, atopic dermatitis, prurigo nodularis, vitiligo, or cutaneous T-cell lymphomas. Targeted photo-

therapy equipment, such as the 308-nm excimer light, is also used. 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.

Self-assessment

Psoriasis: To improve patients' QOL and treatment compliance, we have selected therapies on the basis of their risk/benefit ratios. Phototherapy with narrow-band UVB and the 308-nm excimer lamp has been also applied. Biologic agents, including infliximab, adalimumab, and ustekinumab, have also been used to treat patients with severe psoriasis.

Neurofibromatosis: Many patients with neurofibromatosis type I are still being referred to our special clinic. We are now performing inheritance consultation for pediatric patients. Surgical removal of different types of neurofibroma is performed for inpatient and outpatient clinics to improve QOL. Genetic analysis has been performed for MPNST.

Herpes virus infection: Suppressive therapy has been used to improve impaired QOL. To control PHN, we are prescribing tricyclic antidepressants, serotonin reuptake inhibitors, $Tramacet^{\mathbb{R}}$, other opioid analgesics, and topical analgesics.

Human papillomavirus infections: We have employed new treatments, including topical vitamin D3, contact immunotherapy, and lasers, in addition to ordinary surgical treatments, to treat refractory viral warts. Human papillomavirus typing is also regularly performed.

Contact dermatitis: Patch testing for causal chemicals, environmental allergens, drugs, and foods are regularly performed for patients with contact dermatitis.

Atopic dermatitis: We have been treating patients according to established guidelines and the degree of QOL impairment. The psychosocial background of patients is also considered. To increase patient understanding, we have been organizing atopic dermatitis forums, which include monthly lectures and group meetings. Basic research is focused on pruritogens, such as substance P, IL-31, helper T type 2 including TARC.

Malignant skin tumors: We have been treating many patients with skin cancers, including melanomas, basal/squamous cell carcinomas, and extramammary Paget's disease, with surgical operations combined with sentinel lymph-node biopsies and chemotherapy. At the same time, we have provided supportive care to improve the QOL of patients with incurable conditions.

Laser: We have been treating many patients using several different types of laser. In intractable cases of hemangioma simplex, strawberry mark, and teleangiectasia, we have been able to use the V-beam laser since 2011.

On the basis of many clinical and basic results, it is possible to select appropriate treatments for various aspects of skin diseases in our department.

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Department of Radiology

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

Research Activities

Division of diagnostic imaging

1. A computed tomography scoring system as a predictor of neck metastasis in patients with head and neck cancer

Nodal metastasis is the most important prognostic factor in patients with head and neck cancers. We proposed a computed tomography (CT) scoring system that consists of size, shape, extracapsular spread, and focal defects of lymph nodes. Its clinical applicability was assessed by comparison with surgical specimens of neck dissection.

2. CT 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 CT diagnostic criteria for eosinophilic chronic rhinosinusitis and analyzed their clinical usefulness.

3. CT anatomy of the chorda tympani nerve

The chorda tympani should be identified during the temporal bone surgery with the facial recess approach. We evaluated the normal CT anatomy of the chorda tympani and assessed the clinical significance of preoperative identification of the nerve.

4. Magnetic resonance imaging of ovarian mucinous tumors: Comparison of diagnostic accuracy and intraoperative frozen sections

We compared the diagnostic accuracy of magnetic resonance imaging (MRI) of ovarian mucinous tumors with accuracy of intraoperative frozen sections and explored the role of MRI. Ovarian mucinous tumors tend to be underdiagnosed with intraoperative frozen sections. In contrast, MRI is more capable of suggesting the possibility of borderline and malignant mucinous tumors than are intraoperative frozen sections.

5. Comparison between MRI findings of ovarian serous borderline tumors and ovarian clear cell carcinomas: Mimics on MRI

We retrospectively compared the MRI findings of ovarian serous borderline tumors (SBTs) with those of ovarian clear cell carcinomas (CCCs). A cystic tumor with solid/ papillary components is the major imaging appearance for both SBTs and CCCs. Papillary components are seen more frequently in SBTs than in CCCs, and a papillary architecture with internal branching on T2-weighted images is more specific for SBTs. The apparent diffusion coefficient values of solid portions tend to be lower in CCCs than in SBTs. We conclude that MRI can be used to differentiate SBTs from CCCs. 6. Assessing clinical probability of deep venous thrombosis using the new classing system Contrast-enhanced CT is usually performed to screen for deep venous thrombosis (DVT). If the clinical probability of DVT can be predicted before CT, unnecessary examinations might be avoided. To assess the clinical probability of DVT, we made a new scoring classing based on the clinical data of gynecology patients. We used a machine learning method with a neural network. We evaluated the diagnostic accuracy of the system on the basis of actual clinical outcomes.

This study was performed in collaboration with the Department of Obstetrics and Gynecology.

7. Imaging findings of pulmonary infarction

The clinical symptoms of acute or subacute pulmonary infarction are sometimes atypical, and chest CT might be key for the correct diagnosis. Therefore, understanding the characteristic CT findings and sequential CT changes of pulmonary infarction is important. We reviewed the chest CT images and their sequential changes from 10 patients to clarify useful findings in cases of acute or subacute pulmonary infarction.

8. Chest CT findings of a syndrome positive for antigens against aminoacyl-transfer RNA synthetase

Antigens against aminoacyl-transfer RNA synthetase (ARS) are often positive in patients with polymyositis/dermatomyositis and might involve the lungs. To identify characteristic CT features, we reviewed 22 cases of pulmonary manifestation in patients with anti-ARS antigens from our archives from April 2007 through March 2014.

9. MRI evaluation of the therapeutic effects of biological agents in psoriatic arthropathy Both before and after the start of treatment in patients with psoriatic arthropathy, MR was performed and the presence or absence of enthesitis, synovitis, bone marrow edema, and bone erosion was evaluated. In patients with active psoriatic arthropathy the contrastenhancement effect was present in enthesitis and synovitis. These contrast-enhancement effects disappeared where good therapeutic effects were obtained. Contrast-enhanced MRI is useful for evaluating therapeutic effects in patients with psoriatic arthropathy.

10. Evaluation of frequency of high signal intensity on MRI at the iliacus muscle origin into the iliac fossa

High signal intensity at the iliacus muscle origin enthesis was present in 131 examinations (23.6%) and in 93% of patients older than 80 years. Pathology at the iliacus muscle origin may be an overlooked cause of groin pain and may be associated with development of pathology.

Division of Ultrasound

1. Power Doppler ultrasonography for evaluating the activity of rheumatoid arthritis Power Doppler ultrasonography was performed in the bilateral wrists, elbows, shoulders, knees, and ankles of patients with rheumatoid arthritis. The synovial blood flow signals were scored with a 3-grade scale, and the total score in the 10 joints was regarded as the total signal score. The total signal score was strongly correlated with serum levels of vascular endothelial growth factor, angiopoietin 1, and angiopoietin 2.

Division of Nuclear Medicine

1. Physiological change of accumulation in I-123 iomazenil brain single-photon emission CT during childhood

Physiological regional accumulation of I-123 iomazenil brain single-photon emission CT (SPECT) changes markedly during childhood, especially before the age of 3 years. The aim of this study was to compare regional accumulation in the brain on anatomically standardized I-123 iomazenil brain SPECT images, which were obtained with the 3-dimensional stereotaxic region of interest template, a fully automated software program. A total of 172 patients aged 1 month to 15 years with convulsive disease were examined with iomazenil SPECT in cooperation with Saitama Children's Medical Center; no significant abnormalities were found. We assessed regional accumulation to leverage regional corrected counts/pixel (regional mean counts/pixel/dose administered/patient body surface area) corrected by the time between the measurement of dose and the scan.

In neonates, physiological accumulation was low throughout the brain and was lowest is the frontal lobe, in accordance with cerebral blood flow. As infants aged, accumulation increased in all regions, especially in the occipital lobe and, to a lesser extent, in the cerebellum. The peak iomazenil uptake was in the cerebrum in patients aged 4 to 6 months and in the cerebellum in patients aged 7 to 9 months. The rate of change in physiological accumulation was lowest in the frontal lobe. Decreasing iomazenil uptake in the cerebrum and cerebellum is believed to be related to synapse elimination in the developing cerebrum and cerebellum.

Division of Interventional Radiology

1. Comparing the amount of procedural time and total hemorrhage between temporary balloon occlusion of the common iliac artery and the internal iliac artery during cesarean delivery among patients with placenta previa and placenta accreta

Temporary internal iliac arterial balloon occlusion was previously chosen for cesarean delivery with placenta previa or accureta to prevent massive hemorrhage at The Jikei University Hospital, but the amount of hemorrhage was not satisfactory controlled because of collateral arterial flow.

Therefore, we have changed to temporary occlusion of the common iliac arterial and compared the amount of procedural time and hemorrhage.

We found no significant difference in the amount of hemorrhage. This result may be due to the small number of cases and to the amount of total hemorrhage differing greatly among cases

The procedure time for balloon placement differed significantly (P < 0.05) between the internal iliac artery and the common iliac artery, and anatomical complexity may affect this difference.

No complications occurred in our series, and the common iliac artery occlusion procedure can be more useful in emergency situations because of the short time of balloon placement and may reduce the amounts of radiation exposure and contrast material.

2. Retrospective investigation of percutaneous cryoablation for stage T1b renal cell carcinomas

We investigated clinical outcomes and renal function outcomes of percutaneous cryoabla-

tion performed for stage T1b renal cell carcinomas from September 1, 2011, to July 31, 2014.

3. Percutaneous cryoablation for breast cancer

The inclusion criteria for percutaneous cryoablation for breast cancer are a maximum tumor diameter of less than 1.5 cm, luminal A-like pathological findings of core needle biopsy, the exclusion of ductal invasion with imaging, and the negativity of sentinel lymph node biopsy. We investigated local control and cosmetic outcomes.

4. Efficacy of transcatheter arterial embolization before percutaneous cryoablation for endophytic renal cell carcinomas

We believe that transcatheter arterial embolization provides clear visualization of endophytic renal cell carcinomas during CT-guided procedures. We reviewed the local control, periprocedural complication, and postoperative renal function of patients who had undergone transcatheter arterial embolization before percutaneous cryoablation.

Division of Radiation Therapy

1. Kochi Oxydol-Radiation Therapy for Unresectable Carcinomas (KORTUC) is enzyme-targeting radiosensitization treatment which uses a radiosensitizer, contains hydrogen peroxide at a low concentration, and inactivates peroxidase/catarase in the tumor tissue. Most malignant tumors contain many hypoxic cancer cells and/or large amounts of antioxidative enzymes and are, therefore, resistant to low linear energy transfer radiation. In KORTUC I the superficially exposed tumor is covered with gauze soaked with a hydrogen peroxide solution. In KORTUC II the radiosensitizer is injected into the tumor. A total of 17 patients with locally advanced malignant neoplasms have entered our clinical trial and been treated with KORTUC I or II.

2. Radical radiotherapy for prostate cancer

Prostate cancer has various treatment options. In radiotherapy for prostate cancer, progress has recently been made in ultrahypofractionation. Stereotactic body radiotherapy (SBRT) has attracted considerable attention by allowing the clinical use of ultrahypofractionation. The use of SBRT and intensity-modulated radiotherapy for treating lung cancer has proceeded in our department. We also plan to use SBRT and intensity-modulated radiotherapy to treat prostate cancer.

3. Clinical outcomes of current chemoradiotherapy for esophageal cancer refractory to docetaxel, cisplatin, and fluorouracil

The combination of docetaxel, cisplatin, and fluorouracil (DCF) is a candidate regimen for induction chemotherapy for esophageal cancer, due to its high efficacy. Therefore, the treatment of DCF-refractory tumors is extremely difficult. We evaluated overall survival and the efficacy of locoregional control after concurrent chemoradiotherapy with fluorouracil and cisplatin for DCF-refractory esophageal cancer.

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Department of Surgery Division of Digestive Surgery

Katsuhiko Yanaga, Professor Hideyuki Kashiwagi, Visiting Professor Masahiko Otsuka, Visiting Professor Tetsuji Fujita, Associate Professor Tomoyoshi Okamoto, Associate Professor Yoshio Ishibashi, Associate Professor Satoru Yanagisawa, Associate Professor Shuichi Ishida, Associate Professor Shuzo Kono, Assistant Professor Katsunori Nishikawa, Assistant Professor Shuichi Fujioka, Assistant Professor Yasuro Futagawa, Assistant Professor Katsuhito Suwa, Assistant Professor Katsuhito Suwa, Assistant Professor Hiroaki Shiba, Assistant Professor Kazuhiko Yoshida, Professor Nobuyoshi Hanyu, Visiting Professor Kenji Ikeuchi, Visiting Professor Norio Mitsumori, Associate 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

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.

A boy who underwent surgery at a university hospital died in February 2014 after removal of a cervical lymphangioma due to acute circulatory failure 3 days after he had been transferred to the intensive care unit (ICU) and given propofol intravenously for approximately 70 hours. After the boy's death, the hospital admitted the use of propofol, which was prohibited to be used in children, and that from 2008 through 2013, 11 of their pediatric patients 14 years or younger had died during the treatment or within 30 days after leaving the ICU.

If the surgeons and anesthesiologists who treated the boy in the ICU had read a brief review article and statement in the *Canadian Medical Association Journal*, they would not have treated children with propofol. According to the article, published in 2002, the problem of propofol in children was first noted in 1992, when a report was published about 5 children with croup or bronchiolitis who were sedated with propofol and subsequently in an ICU and died of metabolic acidosis and myocardial failure. Sporadic cases of subsequently termed "propofol syndrome" were described in the literature, including a report in 1998 of 18 critically ill pediatric patients who had bradycardia, asystole, severe metabolic acidosis, lipemia, hepatomegaly, and rhabdomyolysis. Canadian product monographs of propofol infusions are now being updated to indicate that the agent is contraindicated for the sedation of children receiving intensive care.

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): 387-95).

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) expressions 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 are comparing the observation methods of fluorescence imaging and infrared absorption imaging. In addition, we have surveyed immunohistochemical staining and the expression of messenger RNA in tumor cells and evaluated the relationship between these expressions and clinico-pathological findings. Such research revealed that zinc finger protein 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 assessed the stress of surgeons during laparoscopic surgery for colorectal cancer by measuring serum levels of adrenaline, noradrenaline, dopamine, adrenocorticotropic hormone, and cortisol. We are analyzing the data to clarify which factors are related to the stress of surgeons during an operation. We have published the cosmetic outcome of a novel and patient-friendly ileostomy procedure. In this procedure the umbilical fossa is used to place a defunctioning ileostomy, and a simple umbilicoplasty is used for ileostomy closure. A collaborative study with the Department of Urology to identify novel

cancer-related proteins in the gastrointestinal tract is still in progress. The relationships of copy number variation to recurrence and prognosis are evaluated after DNA is extracted from frozen specimens of colorectal cancer tissue, because copy number variation is likely to influence gene function. 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-mvc 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. We will also use a model of liver metastasis through the spleen with a colon carcinoma cell line in mice. Using this model, we plan to analyze factors involved in the development of metastasis. Cancer cells that cause epithelial-mesenchymal transition lose their cellular adhesion and escape into the blood flow by invading blood vessels before reaching the sites of metastasis as circulating cells. We therefore speculate that the control of epithelial-mesenchymal transition inhibits postoperative metastasis to distant sites. We will focus on signal transducer and activator of transcription 3(STAT3) and nuclear factor kappa B(NFKB), which cause inflammation in the cancer microenvironment, and investigate the inhibitory effect of epithelial-mesenchymal transition on recurrence and metastasis.

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 was performed for a patient with primary biliary cirrhosis on June 5, 2015 (15th LDLT). Our 16th LDLT was performed for a patient with nonalcoholic steatohepatitis on July 10, 2015. All 16 recipients were discharged in good condition on postoperative day 15 to 55, and all donors were returned to preoperative status and discharged on postoperative day 7 to 26. 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

76.7%, 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 had its indications gradually 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. Clinical and experimental studies are now evaluating nutritional therapy for patients with cancer, enhanced recovery after surgery, surgical site infection, and the use of eltrombopag before laparoscopic splenectomy for idiopathic thrombocytopenic 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 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 Lapa-

roSonic 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.

Endocrine

1. Basic research

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.

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 antibodies added in vitro. We have investigated the mechanism of cell-to-cell inhibition and association with lymph node metastases.

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Department of Surgery Division of Pediatric Surgery and Vascular Surgery

Takao Ohki, Professor and Chairperson Yuji Kanaoka, Assistant 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

Minimally invasive and scarless surgeries.

a. 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.

b. 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.

c. 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

a. 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 branched 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.

4. Clinical study of specific antibody for heparin-platelet factor 4 complexes

Heparin is commonly used for anticoagulation in vascular surgery. Heparin-induced thrombocytopenia (HIT) is a rare but life-threatening complication with thrombosis of veins and arteries. Even if heparin use is limited, it occasionally induces the production of specific antibodies against heparin-platelet factor 4 (PF4) complexes. Patients with such antibodies are at increased risk for HIT. The prevalence of these antibodies in patients receiving heparin is presumably underestimated. Accordingly, we prospectively measured antibodies against heparin-PF4 complexes and the activity of PF4 and investigated whether they are related to symptoms of HIT, particularly in patients undergoing major vascular surgery. We measured these variables in 300 patients.

The percentage of patients with antibodies to heparin-PF4 complexes was approximately 13%, which was higher than expected. Moreover, PF4 activity tended to be higher in antibody-positive patients than in antibody-negative patients. The results of this study are being statistically analyzed and will be reported.

5. Research on prevention of reperfusion injury during endovascular aneurysmal repair Large sheaths are usually used for endovascular aneurysmal repair. If the inserted sheath is retained at the femoral artery for a long time, the ischemic time of the lower extremities becomes longer, and reperfusion syndrome might occur. We have inserted a small sheath into the distal side of the femoral artery and created a shunt to supply blood flow to the distal lower extremities and to prevent complete ischemia of the lower extremities and consequent reperfusion syndrome.

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Department of Orthopaedic Surgery

Keishi Marumo, Professor Hajime Sugiyama, Professor Shigeru Soshi, Associate Professor Mamoru Yoshida, Associate Professor Yutaka Ueno, Assistant Professor Takuya Otani, Professor Hiroki Funasaki, Associate Professor Makoto Kubota, Associate Professor Mitsuru Saito, Associate Professor Hideki Fujii, 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 has been focused on the relationship between osteoporosis and the risk of fracture. We have shown that high levels of pentosidine in urine or blood and mild hyperhomocysteinemia, which suggest bone collagen abnormalities, may be used as surrogate markers for evaluating bone quality and assessing the risk of fracture. Our clinical research focuses on the relationship between systemic disorders, such as lifestyle-related diseases and aging, and bone disease. In basic research, we analyze hard tissue characteristics in a mouse model of mucopolysaccharidosis, in collaboration with the Department of Pediatrics, and in epigenetically modified giant cell tumors, in collaboration with the Department of Laboratory Medicine.

Our studies of β -tricalcium phosphate (β -TCP) have played a pioneering role in the field of bone grafting. They have led to the wide application of β -TCP in many clinical settings; e.g., due to its efficient bone formation profile, β -TCP has been used as a complementary filling material in repair of bone defects.

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, 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 spine team has demonstrated the effectiveness of early open-door laminoplasty with hydroxyapatite block spacers and lamina plate. The lamina plate provides more rigid fixation than does hydroxyapatite block. The use of this novel lamina plate may help minimize complications and allow patients to engage in more aggressive rehabilitation programs. The technique might reduce both the incidence of postoperative axial neck symptoms and the loss of motion associated with traditional laminoplasty. The spine team surgeons have been investigating surgical outcomes of patients who had undergone laminoplasty with the developed technique.

The knee joint team has been performing total knee arthroplasties with patient-matched instrumentation and analyzed the effectiveness of cutting-edge technologies even more advanced than the surgical navigation system itself.

Through the wide range of clinical research activities, all teams fulfill their important
clinical, scientific, and educational roles at our academic hospital, and their commitment is highly valued.

Research Activities

Sport activities following nonoperative treatment of full-thickness rotator tears in elderly sports enthusiasts

We performed a retrospective study of the results of nonoperative treatment in elderly sports enthusiasts who had full-thickness tears of the rotator cuff. The subjects were 35 patients (with 37 shoulders) with a mean age of 63 years. The size of the cuff tear was small in 8 patients, median in 19, global in 6, and massive in 4. The mean time of follow-up was 28 months.

The mean Japanese Orthopaedic Association score at the patient's first visit was 67 points. All patients quit their sports activities after the injury. At the final follow-up examination, the mean score had improved to 91 points and was correlated with the tear size. Although all patients returned to their previous sport activities, the Japan Shoulder Society Shoulder Sports Score had a mean value of 84 points and was also correlated with the tear size. Scores were low in patients with large or massive tears and patients who played tennis or swam.

Clinical and research activities of the hand surgery team

We treat many kinds of disease from trauma (fractures, tendon ruptures, and neurovascular injuries) to degenerative diseases and tumors. We also provide specialized surgical techniques for suturing tendons and microsurgery. Last year we performed more than 300 operations. After surgery, we cooperate with occupational therapists in the outpatient clinic to help patients achieve full functional recovery. Our clinical research focuses on collagen cross-linking in the hands of patients with Dupuytren contracture to clarify the etiology of this disease. Furthermore, we analyze risk factors of postoperative complications after enucleations of schwannomas.

Open-door laminoplasty with novel plate spacers

Laminoplasty is the most commonly performed surgical treatment for cervical compressive myelopathy. This conventional approach has complications, such as cervical axial pain and loss of neck range of motion. The open-door laminoplasty with only hydroxyapatite block spacers does not provide enough rigid fixation; however, with plates, more rigid fixation can be obtained. Using our newly designed plate for laminoplasty may help minimize complications and allow patients to engage in a more aggressive postoperative rehabilitation program. Our technique might decrease both the incidence of postoperative axial neck symptoms and the loss of motion currently associated with conventional laminoplasty.

Femoral reconstruction with modular and interlocking stems in revision total hip arthroplasty

We have adopted cementless reconstruction and used 2 stem-based reconstructions with a

modular system or with an interlocking stem. The objectives were to evaluate short-term results of femoral reconstructions with the adopted stems. A total of 120 revision total hip arthroplasties were examined. The complication rate was higher in the modular stem group, which had all 13 femoral fractures. The modular stem group also showed a higher rate of successful bone ingrowth (100%) than did the interlocking stem group (12%). Furthermore, femoral reconstruction was frequently complicated by fractures in the modular stem group; however, biological fixation was achieved, and good long-term results could be expected. The interlocking stem can be safely used with a low incidence of complications, and excellent short-term result can be expected. However, proper biological fixation is difficult to achieve.

Computed tomography-based navigation-assisted total knee arthroplasty: Outlier analysis

The purpose of this study was to analyze differences in the outliers' data between computed tomography (CT)-based navigation-assisted total knee arthroplasty and conventional technique arthroplasties. We retrospectively compared the alignment of 130 total knee arthroplasties performed with a navigation system with that of 67 arthroplasties done with a conventional system. The mean preoperative leg axis of 10 outliers in the navigation group was $199^\circ \pm 3.1^\circ$ and that of 17 outliers in the conventional group was $192^\circ \pm 9.7^\circ$. The femoral component of the outlier cases in the navigation group was placed in the varus position.

We specified characteristics of the outliers and used postoperative radiographs to examine the accuracy of the CT-based navigation system. Our results suggest that the CT-based navigation system requires further improvements in accuracy.

The mechanism by which systemic glucocorticoid administration causes weakening of the Achilles tendon

Our study aimed to determine the mechanism by which systemic glucocorticoid administration causes weakening of the Achilles tendon. We used a mouse model to evaluate quantitative and qualitative changes in collagen. In the glucocorticoid group the maximum tensile load and the gene expressions of type 1 collagen and lysyl oxidase were decreased and the content of enzymatic collagen crosslinks was significantly lower. The corresponding amount of senescent crosslinks did not differ. The mean collagen fiber diameter was smaller. Our observations suggest that systemic glucocorticoid administration reduces the strength of the Achilles tendon by decreasing its collagen content, hindering formation of enzymatic crosslinks, and thereby causing collagen to remain in its immature state with smaller fiber diameters.

Clinical and research activities of the bone metabolism team

In our outpatient clinic specializing in bone metabolism, we provide individualized care with simultaneous estimation of bone quantity and quality. We treat several types of refractory bone disease, such as Paget disease, osteomalacia, and osteoporosis that is postmenopausal, induced by glucocorticoid, or associated with a hormonal disorder, childbirth, or vegetarianism.

Our clinical research is focused on the relationship of bone disease to systemic disorders, such as lifestyle-related diseases, and aging. Analyses of bone quality in patients with chronic obstructive pulmonary disease or nonalcoholic steatohepatitis are now in progress. In basic research, we analyze hard tissue characteristics in a mouse model of muco-polysaccharidosis, in collaboration with the Department of Pediatrics, and in epigenetically modified giant cell tumors, in collaboration with the Department of Laboratory Medicine.

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Department of Neurosurgery

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

General Summary

The research studies in our department, examining such topics as syringomyelia, endovascular surgery, mechanism of head injury, and pediatric neurosurgery, made good progress 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. We are now analyzing the data and aim to publish these data in several neurosurgical journals.

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

Owing to research in collaboration with the Tokyo University of Science, we have been making numerous contributions regarding the biofluid mechanics of brain aneurysms using computational fluid dynamics analysis. The research collaboration has been steadily expanding, and several international collaborative studies are now in progress. 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 novel imaging software for the analysis of cerebrovascular disease To improve the image quality of current modalities, e.g., magnetic resonance, computed tomography (CT), and angiography, several types of image-processing software are under development. The prototype of a novel software program to remove metal artifacts from C-arm CT images has been installed in our animal laboratory. By significantly reducing artifacts due to metal coils, this software has significantly improved visualization near the coil mass in C-arm CT images. The results of the data analysis were recently published in the *American Journal of Neuroradiology* (in press).

The software has recently become commercially available for angiography devices built by Siemens Medical Systems (Erlangen, Germany). The clinical data is now being investigated and will be reported some time 2015.

Other software programs, such as *syngo* PBV Neuro (for measuring cerebral blood volume during angiography), and a high-resolution C-arm CT are also commercially available. The collected data is now being analyzed, and the results are expected to be reported at the annual conference of the Neurosurgical Association and the Japanese Society of Neuroendovascular Therapy in 2015.

A new software program called 4-dimensional digital subtraction angiography has recently become available for angiography workstations. The new software provides high spatial resolution and temporal resolution at the same time in a single acquisition. We have already performed this software for more than 60 patients and discovered that it is extremely useful, especially for patients with vascular malformation, for example, arteriovenous malformations and dural arteriovenous fistulas.

4. 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. The novel stent device has a very low profile delivery system (compatible with a 2.1-Fr system) and functions as a flow-diverter device but can still be used for stent-assisted coil embolization.

A preclinical animal study is in progress. This project is supported by a research grant from the Ministry of Economy, Trade and Industry for more than \$50 million over 5 years. 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 February 2016.

5. Development of novel bioactive coil device for the treatment of brain aneurysms

The Matrix Detachable Coil System was introduced to the market in 2002 as a first-generation bioactive coil material for treating aneurysms. This device has been used for more than 70,000 patients throughout the world, and now a second generation of bioactive coils is being developed. The results of animal experiment have been promising, and preclinical animal studies are now in progress.

6. Establishment of a telemedicine network utilizing a novel software for smartphones After the successful introduction of the mobile telemedicine software program "i-stroke," the quality of stroke care in our institution has been dramatically changed. Now, "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 73rd annual meeting of the Japan Neurosurgical Society and the 32st annual meeting of the Japan Society for Neuro-Oncology, we reported that cotransfection of Poly(I:C) and IL-10 siRNA into fusions of DCs and tumor cells is a practical strategy to enhance antitumor responses. The FC immunotherapy is now in a process to receive approval from the Ministry of Health, Labor and Welfare as an advanced medical treatment.

2. Establishment of the Brain Tumor Bank

We established a system to preserve the tissue of brain tumors with a refrigeration named the Brain Tumor Bank. The frozen tissues will be analyzed with a recently developed next-generation DNA sequencing system that allows us to sequence DNA and RNA much more quickly and inexpensively than with the previously used Sanger sequencing system. These studies will help develop a new diagnostic procedure and novel therapy in the future.

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.

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.

Spine and spinal cord group

Numerous conditions, including syringomyelia, degenerative spine diseases, spinal cord tumors, and spinal vascular lesions, have been major concerns of our department. The departments of orthopedic surgery and neurosurgery often collaborate in the interests of patient-oriented treatment in our hospital.

In clinical research, an analysis of pain in patients with neuropathic pain was started. The DynaCT scanning system (Siemens Medical Systems) in operating rooms 4 and 5 is one of the most sophisticated image-guided surgery systems, especially when paired with a navigation system.

Basic research, including research on spinal cord injury and regeneration technology, has just begun in our group.

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.

Our clinical research for craniosynostosis surgery, in collaboration with the Department of Plastic and Reconstructive Surgery, has developed operative procedures using distraction method depending on different age and has received the honorable prize of the International Society for Pediatric Neurosurgery, Raimondi's Award, in 2004 and the Kawabuchi Award in 2005.

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Department of Plastic and Reconstructive Surgery

Mitsuru Uchida, Professor Shintaro Matsuura, Associate Professor Kimihiro Nojima, Associate Professor Katsuhiro Ishida, Assistant Professor Takeshi Miyawaki, Associate Professor Kunitoshi Ninomiya, Associate Professor Junya Hayashi, Assistant Professor Katsuya Mori, Assistant 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 may compromise the shape of the nasal pyramid. Recently we have been collaborating with otorhinolaryngological surgeons in functional rhinoplasty and have introduced open septorhinoplasty techniques that are widely used in aesthetic surgery. The open approach allows correction of the deviated L-strut under direct vision and 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 columella 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 fracture, comminuted fracture, fracture adjacent to the joint), joint contractures, malunion of fractures, and pathological fractures caused by enchondroma. It was also used with good results in bone lengthening and the temporary traction of joints. Use of the Ilizarov minifixator is an effective and noninvasive method and is highly recommended for selected cases.

A long-term follow-up study of Apert hand

The treatment of hand deformity in Apert syndrome is challenging. Digital separation is usually completed before 2 years of age to ensure proper growth and functional development; however, few studies have investigated the long-term results of digital separation. We described long-term results of digital separation performed in our department to show how Apert hand is improved functionally by surgical treatment. Forty-two patients were treated from 1974 through 2013; 5 of these patients were followed up for more than 10 vears after complete digital separation. Evaluation included the range of motion in the hands, radiographic examination, and the Disabilities of the Arm, Shoulder, and Hand Ouestionnaire score. According to Upton classification, the hand deformity was type 1 in 1 patient and was type 2 in the other patients. Three of the 5 patients who were followed up long term underwent mental development testing, which showed IOs of 57, 70, and 92. Most interphalangeal joints were stiff, and the total active motion of the fingers ranged from 0 to 65 degrees. Radiographs showed fused interphalangeal joints. The Disabilities of the Arm, Shoulder, and Hand Questionnaire scores ranged from 4 to 32, with good scores for the 2 patients with IQs greater than 60. Some of the patients can write with a pen, eat with chopsticks, and type on a keyboard. None of the patients were able to pinch between thumb and fingers; however, some of the patients pinched between index and middle fingers or the middle and ring fingers to pick up small objects. None of the patients were employed. Digital separation of Apert hand contributes to functional and aesthetic improvement, but further evaluation is needed to understand the long-term affects of Apert hand on patients, especially those of normal intellectual ability.

Free skin 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 free skin flap transfer after partial hypopharyngectomy with laryngeal preservation. The subjects were 54 patients who underwent free skin flap reconstruction immediately after partial pharyngo-laryngectomy 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. Fortythree patients (80%) were able to eat an unrestricted diet and experienced no aspiration. Restriction of the diet was significantly correlated with the extent of esophageal mucosal resection. Free skin 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 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 POS-SUM 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.

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Department of Cardiovascular Surgery

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

General Summary

The major achievements in our department included both clinical studies and experimental animal studies. The clinical studies include those establishing excellent surgical performance, investigating new techniques, and evaluating alterations in cardiac performance and long-term results after cardiac surgery. The experimental animal studies are performed to address clinical problems we are facing. A recent topic for adult surgery is the introduction of new fields, a transcatheter aortic valve replacement, and a left ventricular (LV) assist device program. We are preparing for them by having continuous meetings. We are also continuously performing several experimental studies with in-vivo models. The experimental projects include evaluation of hemodynamic performance during the Glenn and Fontan procedures, protection of the heart during cardiac arrest, and pulmonary valve function. A new project we started this year is visualization of the cardiac conduction system in human heart specimens with high-resolution phase contrast computed tomographic (CT) imaging. The major activities are described below.

Research Activities

Experimental studies of "remote per-conditioning" as a new therapeutic strategy of myo-cardial protection

An experimental study in an in-vivo piglet model was performed to test the hypothesis that ischemia/reperfusion (I/R)-induced biochemical damage and LV dysfunction can be reduced by "remote per-conditioning" (intermittent I/R of a remote organ before myocardial reperfusion). Fifteen piglets underwent 120 minutes of ischemia followed by 60 minutes of reperfusion while on cardiopulmonary bypass (CPB). In 5 of the piglets, remote ischemic preconditioning with 3 cycles of 30 seconds of I/R of a lower limb were applied before aortic unclamping, whereas the other piglets were not treated. Systolic and diastolic dysfunction of the LV associated with oxidant-induced biochemical injury was noted in the untreated group. In contrast, per-conditioning allowed significantly better LV functional recovery and less myocardial biochemical injury. This study in a piglet CPB model suggests that "remote per-conditioning" produces prompt myocardial functional recovery with less biochemical injury. Experimental studies of the severity of the pulmonary regurgitation fraction: Variability of pulmonary regurgitation in proportion to pulmonary vascular resistance in a porcine model of total resection of the pulmonary valve

We investigated the effect of the physiological changes in pulmonary vasculature and right ventricular function on the hemodynamic severity of pulmonary regurgitation (PR) in a porcine model with severe pulmonary regurgitation.

Pulmonary vascular resistance (PVR) was changed by manipulating the $PaCO_2$ and by the inhalation of nitric oxide, and right ventricular (RV) function was manipulated with a dobutamine stress test to verify the effect of PVR and RV systolic function on the pulmonary regurgitant fraction (PRF). We found a significant positive correlation between PRF and PVR and a negative correlation between PRF and RV-segment shortening. This study demonstrates that PRF varies in proportion to changes in PVR and RV systolic function, which indicates that low PVR and high RV contractility are advantageous in reducing the severity of PR and the RV volume load after RV outflow tract reconstruction.

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

Introduction: The feasibility of visualization of the atrioventricular (AV) conduction axis in human whole heart specimens by means of a synchrotron radiation phase-contrast CT (PCCT) was tested.

Method. The PCCT images of 4 formalin-fixed autopsy specimens of normal whole hearts were collected in the Biomedical Imaging Centre, Super Photon Ring 8 Synchrotron Facility (Hyogo, Japan) with a PCCT system consisting of a Talbot interferometer. In the resliced PCCT images, low-density structures were distinctly recognized from surrounding tissue. Such structures were detected in the AV septum, penetrating the central fibrous body, extending on top of the interventricular septum, and bifurcating onto both sides of the ventricular septum. These structures were comparable with the AV conduction axis on pathological observation. From images sequenced, major subdivisions of the AV conduction axis were visualized within 3-dimensional heart structure.

Conclusion: 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 studies of surgical outcomes of patients with univentricular heart

1. Effect of pulmonary vasodilators on the pulmonary circulation in patients waiting for Fontan procedure following the Glenn operation

We retrospectively studied sequential changes of the pulmonary circulation and the effect of pulmonary vasodilators in patients waiting for Fontan procedure following the Glenn operation.

Thirty consecutive patients with single ventricle physiology were selected (double-outlet right ventricle, 11 patients; mitral atresia, 5 patients; tricuspid atresia, 4 patients; transposition of the great arteries, 4 patients; pulmonary atresia with intact ventricular septum, 2 patients; and other, 4 patients). The mean age of patients during the Glenn operation was 12 ± 9 months, the mean waiting period for the Fontan procedure was 16 ± 10 months, and the mean number of times the cardiac catheterization was performed was 2.8 ± 1.2 . One or more pulmonary vasodilators (sildenafil, 3 mg/kg/day; tadalafil, 1 mg/kg/day; bosentan, 3 mg/kg/day; and ambrisentan, 0.2 mg/kg/day) were administrated to 15 patients starting from immediately after the Glenn operation. The other 15 patients served as a control group. In the control group, no significant change in pulmonary circulation or vascular features was observed while waiting for Fontan procedure. On the other hand, 6 to 12 months of pulmonary vasodilator therapy significantly decreased pulmonary artery (PA) pressure (PAP) by $85\% \pm 5\%$ and pulmonary resistance by $86\% \pm 7\%$ (p < 0.05). Our data suggest that treatment with pulmonary vasodilators for high-risk Fontan case might shorten the waiting period for Fontan procedure following Glenn operation by improving PAP and pulmonary resistance.

2. Optimal timing of the bidirectional Glenn procedure to avoid interstage drop-out before the final Fontan procedure

The effects of age when the bidirectional Glenn procedure is performed and of preoperative characteristics on the incidence of inadequate Fontan candidacy were analyzed with univariate/multivariate logistic regression in 49 patients who underwent the Glenn procedure (2001-2014). Impaired Fontan indication criteria were defined as PAP \geq 15 mm Hg or PVR index \geq 3.0 Wood units for the pulmonary factor and systemic ventricular enddiastolic pressure \geq 12 mm Hg for the ventricular factor. Multivariate regression analysis revealed that the presence of additional PA flow and the age when the bidirectional Glenn procedure was performed were independent predictors for impaired pulmonary and ventricular criteria, although preoperative hemodynamic variables and other anatomical subsets were not significant predictors on univariate regression. The incidence of impaired pulmonary and ventricular criteria was significantly lower in patients who underwent the bidirectional Glenn procedure before 12 months and 8 months, respectively, than in older patients. In conclusion, the optimal timing of the bidirectional Glenn procedure is when the patient is younger than 12 months for pulmonary risk factors and younger than 8 months for ventricular factors.

3. Validity of extended indication of fenestration to borderline cases

Since 2001, the indications for fenestration at our institution have been extended to the lower-risk patients, who had at least 1 of the following criteria: PAP >15 mm Hg, peripheral resistance > 3.0 Wood units, PA index < 150 mm²/m² body surface area, systemic ventricular dysfunction, associated procedures, history of multiple open palliation procedures or Fontan take-down procedures, and use of a pulmonary vasodilator.

Consequently, of all patients undergoing the Fontan procedure, 18 patients underwent fenestration of 4 mm. Among these patients, 12 had spontaneous closure within 1 to 2 years after the Fontan procedure, whereas fenestration remained patent in the other 6 patients, including 4 patients with fenestration-dependent circulation. Postoperative catheterization revealed that patients with patent fenestration had higher central venous pressures, lower cardiac indexes, and higher levels of type IV collagen, as a marker of liver fibrosis, even with fenestration, than did patients not undergoing fenestration or patients with spontaneous closure. On the basis of these findings we conclude that extension of

the indications for fenestration to borderline cases appears to be an appropriate strategy to facilitate Fontan adaptation in low-risk cases leading to natural closure and to reduce morbidity and late complications in high-risk cases with ensured persistent patency.

Clinical study of adult cardiac surgery

1. Choice of aortic valve prostheses in the rapidly aging society

The current Japanese guidelines recommend the use of a tissue valve for aortic valve replacement (AVR) in patients older than 65 years. We have chosen an aortic prosthesis according to the guidelines after discussions with patients and family about various factors, such as age, comorbidities, surgical, and reoperation risk. Although the use of aortic tissue valves has markedly increased with the recent progression of the aging society, the detailed explanation of valve durability and anticoagulation therapy causes most patients to allow the type of valve to be chosen with the surgeon's experiences and preferences. Clinicians have often changed their ideas of which valve is best for patients aged 60 to 70 years, because patients live increasingly longer than the valves remain improvingly durable and will therefore require a new valve in 10 or 20 years. In the present study, we used propensity score matching to analyze the clinical outcomes after AVR in the recent rapidly aging society, focusing on the choice of valve for patients aged 60 to 70 years. From April 1995 through March 2014, 366 adult patients underwent single AVR or combined AVR/coronary artery bypass grafting. Of these patients, 127 (35%) received a mechanical valve and 239 (65%) received a tissue valve. A retrospective analysis of the entire population and the selected 124 patients aged 60 to 70 years was performed in comparison between the tissue valve and mechanical valve groups. Perioperative deaths occurred of 4 patients in the mechanical valve group (3.1%) and 8 patients in the tissue valve group (3.3%; P = 0.92). The 16-year overall survival rate was $87\% \pm 4\%$ for the mechanical valve group and $40\% \pm 29\%$ for the tissue valve group (p = 0.220). The rate of freedom from reoperation at 16 years was $98\% \pm 2\%$ for the mechanical valve group and $82\% \pm$ 9% for the tissue valve group (p = 0.005). Propensity matching of the patients aged 60 to 70 years did not identify significant differences in the 16-year survival rate and freedom from reoperation between the tissue valve group and mechanical valve group. Both physicians and patients consider the possibility of reoperation in their 80s to be more serious than the risk of anticoagulant-related complications. The environment using tissue valves for patients aged 60 to 70 years has not been appropriate in the society that is rapidly aging and has an increased lifespan.

2. The fundamental technique of mitral valve repair in active infective endocarditis Many institutions have reported good results with mitral valvuloplasty (MVP) for active infective endocarditis (AIE) in the mitral position. However, the reparability of mitral regurgitation due to AIE ranges from 35% to 81%. These reports have not shown the surgical strategies or the definition of AIE. The aim of this study was to examine the results of MVP for AIE during the last decade and to assess the surgical outcomes, with a focus on the validity of MVP for AIE. From January 2004 through December 2014, a series of 32 consecutive patients underwent MVP for AIE in the mitral position. The patients' mean age was 60 years. The infected portions were anterior mitral leaflet in 20 cases and posterior mitral leaflet in 27 cases. In 15 cases the infected lesions were in both the anterior and posterior mitral leaflets. In 7 patients the infected area was in the annulus. Of these 32 patients, 27 underwent MVP and 5 underwent mitral regurgitation. The used techniques were of 5 types: leaflet resection, patch repair, artificial chordoplasty, edge-to-edge repair, and ring annuloplasty. For most cases of AIE, MVP is a useful treatment and can be achieved with radical resection of infected portions and coverage of defects with a pericardial patch supported by artificial chordae. For cases with wide invasion or destruction, which should be resected, more complex procedures are needed. Therefore, early surgical intervention should be considered for successful MVP and for higher survival rates.

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Department of Obstetrics and Gynecology

Aikou Okamoto, Professor Hiroshi Sasaki, Professor Shigeki Niimi, Associate Professor Hirokuni Takano, Associate Professor Osamu Samura, Associate Professor Kouhei Sugimoto, Assistant Professor Nozomu Yanaihara, Assistant Professor Kazuhiko Ochiai, Professor Seiji Isonishi, Professor Kuniaki Ohura, Associate Professor Kyosuke Yamada, Associate Professor Satoshi Takakura, Assistant Professor Hiroshi Tanabe, Assistant Professor Motoaki Saitou, 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. Antitumor effects of the interleukin 6/interleukin 6 receptor signaling pathway inhibition in clear cell carcinoma of the ovary

We aimed to clarify whether the interleukin 6 (IL-6)/IL-6 receptor (IL-6R) mediated signaling pathway has clinical relations with clear cell carcinoma of the ovary (CCC) and to evaluate the inhibitory effects of the pathway on CCC carcinogenesis. We found that the IL-6/IL-6R signaling pathway acts on CCC cells to enhance invasion and chemoresistance and that targeting the IL-6/IL-6R mediated signaling pathway could be a promising therapeutic strategy for CCC.

2. Somatic copy number alterations associated with Japanese patients or endometriosis in ovarian CCC

We used high-resolution comparative genomic hybridization to identify somatic copy number alterations associated with the clinical characteristics of CCC. We found that possible associations between CCC and amplification of the zinc finger protein 217 gene (*ZNF217*) among Japanese patients and between endometriosis and amplifications of the epidermal growth factor receptor gene (*EGFR*).

3. The microRNA 21 gene is a candidate driver for 17q23-25 amplification in ovarian CCC

The aim of this study was to investigate the role of the microRNA 21 gene (miR-21) in 17q23-25 amplification associated with CCC oncogenesis. We found that miR-21 is a possible driver gene other than *PPM1D* (protein phosphatase, Mg2+/Mn2+ dependent 1D gene) for 17q23-25 amplification in CCC. Aberrant expression of miR-21 by chromosomal amplification might play an important role in CCC carcinogenesis through the regulation of the *PTEN* (phosphatase and tensin homologue) tumor suppressor gene.

4. Dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 regulates epithelial-

mesenchymal transition through Snail degradation in ovarian serous adenocarcinoma The aim of this study was to clarify whether dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 (DYRK2) regulates epithelial-mesenchymal transition through Snail degradation in ovarian serous adenocarcinoma. Immunohistochemical analysis demonstrated that DYRK2 expression inversely correlated with Snail expression, and reduced expression of DYRK2 was associated with shorter overall survival in serous adenocarcinoma. DYRK2 may regulate epithelial-mesenchymal transition through Snail degradation in serous adenocarcinoma.

5. Laparoscopic myomectomy with a new suturing-training instrument: 4 case reports

We report 4 cases in which laparoscopic myomectomy was performed after we trained with a suturing box model we developed. We made the model with a stuffed toy that was similar in shape to the uterus and penetrated it longitudinally with wire. Our box model is useful for training in 3-dimensional suturing and the introduction to laparoscopic myomectomy.

6. Clinicopathological examination of AT-rich interactive domain-containing protein 1A expression in stage I ovarian CCC

This study sought to clarify the clinicopathological significance of the loss of AT-rich interaction domain 1A (ARID1A) expression in stage I ovarian CCC. We found that the loss of ARID1A function contributes to the initial stages of carcinogenesis in ovarian CCC and to tumor progression in ovarian CCC derived from endometrial cysts, particularly when tumor cells appear in ascites fluid.

7. Treatment-interval associated effect of irradiation on locoregionally relapsed ovarian cancer

We reviewed clinical records of 61 patients with epithelial ovarian cancer who had received irradiation after chemotherapy was repeated from 1997 through 2006. We showed that irradiation for recurrent ovarian cancer produced a greater survival benefit when applied to chemo-responsive, locoregional recurrent tumor immediately after chemotherapy.

8. Clinical experience of J-VAC drain for skin closure in the laparotomy of obstetrics and gynecology

This study revealed that the subcutaneous J-VAC silicone drain (Ethicon Endo-Surgery Inc., Johnson & Johnson) is useful for closing surgical incisions in gynecology and obstetrics and has no limitations for applicability.

9. Granulosa cell tumors of the ovary: A clinicopathological study of 56 patients

The objective of this study was to investigate the clinicopathological characteristics and the factors related to disease recurrence in ovarian germ cell tumors (OGCTs). This study suggests that OGCTs require long-term follow-up. Because a residual tumor was the risk factor for the recurrence of adult-type OGCTs, it is important to observe the abdominal cavity carefully and achieve complete resection of the tumor during surgery.

10. Ovarian serous borderline tumor: A clinicopathological analysis of 43 cases

The aim of this study was to clarify clinicopathological features associated with malignant potential in ovarian serous borderline tumors (SBTs). Because an SBT with either bilateral occurrence or exophytic growth tends to have extraovarian implantation, these growth patterns may be important as prognostic factors for SBTs. In addition, the activation of the V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS)/B-Raf protooncogene, serine/threonine kinase (BRAF) protein kinase pathway in SBTs with micropapillary pattern could be a possible reason for SBTs to progress toward low-grade serous carcinoma.

11. Innovation of reduced port surgery applying the modified Higuchi's transverse incision

We introduced low single-incision laparoscopic surgery for cystectomy and myomectomy using Higuchi's transverse incision procedure. Low single-incision laparoscopic surgery was suggested to be effective for the treatment of large tumors and to be as safe as conventional procedures.

Perinatology

1. Establishing possible connections among antenatal stress, anxiety, and postpartum depression using human herpesvirus 6 and 7 or antibodies

The Department of Virology of The Jikei University School of Medicine has identified human herpesvirus (HHV) 6 and HHV-7 as potentially effective ways to measure levels of stress and fatigue. The purpose of this study was to establish possible connections among antenatal stress, anxiety, and postpartum depression using HHV-6 and HHV-7 or antibodies found in maternal blood and sputum for evaluation on a depression scale. As of July 2015, we have succeeded in enrolling 139 pregnant women and are now in the process of analyzing the received data.

2. The importance of prenatal genetic counseling

We performed tests to detect an euploidy in high-risk pregnant women with adequate genetic counseling in Japan. The clinical data, test results, and pregnancy outcomes were recorded. Our study describes the findings of a nationwide demonstration project in Japan aiming to introduce noninvasive prenatal testing for fetal an euploidies using maternal plasma, based on the historical background of the prenatal diagnosis. We expect our data to stimulate a debate regarding prenatal genetic testing and hopefully lead to improvements in the perinatal care system with respect to genetic counseling in Japan.

3. Immunohistochemical localization of bilirubin oxidation in human placenta

Bilirubin is an intrinsic antioxidant, produced from heme through biliverdin, which is catalyzed by heme oxigenase 1 (HO-1) and generates oxidative metabolites called biopyrrins as a result of the reaction with reactive oxygen species. To find a new biomarker of pathological pregnancy, in this study we investigated the expression of these enzymes in the human placenta. Placental tissues from 10 patients with pre-eclampsia and 7 patients with uncomplicated preterm deliveries were examined immunohistochemically with monoclonal antibodies against bilirubin (24G7) and HO-1 (EP1391Y). Although staining for HO-1 did not differ significantly between the groups, staining for bilirubin was more diffuse and more intense in cases of pre-eclampsia than in uncomplicated cases. This results suggest that biopyrrin will be a useful biomarker to predict pathological pregnancy relating to oxidative stress.

Reproductive endocrinology

1. Agenda for starting clinical practice of Oncofertility

Fertility preservation for cancer survivors is emphasized with advancing of both cancer treatment and reproductive medicine. We researched the actual circumstance and agenda of oncofertility by examining the patients preserving their sperms or embryos. The results showed the patients after receiving chemotherapy are decreasing, and the unmarried patients who preserve their sperms are increasing. It indicated that the issue of oncofertility was being recognized gradually after Japan Society for Fertility Preservation was established in 2012. Cancer treatment should be taken on top priority in Oncofertility. We should provide information and take care of patients fully by multidisciplinary support team when examining immediately fertility preservation. We consider it important that we should construct the fully coordinating system between each facilities or each professional.

2. The Impact of Word "Oocyte Aging" on Infertility Patients

The expression "oocyte aging" was used in a special news report which made a big impact on society. The impact of "oocyte aging" also influenced the content of infertility counseling. The number of infertility counseling sessions associated with the key words of "termination of treatment" and, "infertility in aging women" markedly increased. Now, we need to discuss female fertility decline with advancing age. In the future, we should consider when and how to provide information about female fertility decline with advancing age. Public education about female fertility decline with advancing age need to decrease the number of aging infertile women desiring children, and must be effective at reversing the decline in the nation's birth rate.

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Department of Urology

Shin Egawa, Professor Koichi Kishimoto, Professor Koji Asano, Professor Yasuyuki Suzuki, Associate Professor Kenta Miki, Assistant Professor Takahiro Kimura, Assistant Professor Shoichi Onodera, Professor Hiroshi Kiyota, Professor Nozomu Furuta, Associate 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) Analysis of the mechanism of cross-sensitization between the colon and bladder via stimulation of the transient receptor potential cation channel ankyrin 1 receptor in the colon or bladder in rats

5) Reduced expression of estrogen receptor alpha in the bladder of patients with interstitial cystitis or bladder pain syndrome

2. Clinical research: Several clinical studies are on going 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) Study of deep venous thrombosis after urological surgery

2) Prospective study of the efficacy of the sacral epidural block versus the pelvic plexus block for transrectal prostate needle biopsy

3) Clinical study of sentinel lymph-node dissection for prostate and bladder cancer

4) Search of biomarkers for detecting interstitial cystitis/bladder pain syndrome

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Department of Ophthalmology

Hiroshi Tsuneoka, Professor Hisato Gunji, Associate Professor Satoshi Nakadomari, Associate Professor Masaki Yoshida, Associate Professor Akira Watanabe, Associate Professor Takaaki Hayashi, Assistant Professor Takuya Shiba, Assistant Professor Yoichiro Masuda, Assistant Professor Keigo Shikishima, Professor Genichiro Takahashi, Associate Professor Kazushige Toda, Associate Professor Tadashi Nakano, Associate Professor Tsutomu Sakai, Assistant Professor Katsuya Mitooka, Assistant Professor Koichi Kumegawa, Assistant Professor Yoshiaki Kabata, 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 (fMRI), glaucoma, electrophysiology, diabetes, vitreoretinal diseases, age-related macular degeneration, uveitis, color vision, and the cornea.

Research Activities

Cataract

The widespread use of ultrasound technology in cataract surgery and the introduction of foldable intraocular lenses (IOLs) have allowed cataract surgery and IOL implantation through incisions of 2.4 to 3.0 mm. Surgeons are now experimenting with even smaller incisions. We began to use a standard phacoemulsification and aspiration device to perform bimanual phacoemulsification and aspiration with a sleeveless phaco tip through an incision 1.2 to 1.4 mm wide. We used an irrigating hook through a side port to infuse the anterior chamber. After the lens was extracted, we were able to safely implant hydrophobic acrylic single-piece IOLs through a 1.8-mm incision. We are able to choose various premium IOLs, for example, multifocal IOLs, toric IOL, and yellow IOLs. We implant these new IOLs and evaluate subsequent visual function.

Neuro-ophthalmology

1. We edited the textbook entitled *Clinical Decisions in Neuro-Ophthalmologic Disorders* and wrote the book's chapters about optic disc edema being a medical emergency, optic neuritis in children, neuroretinitis, and papilledema.

2. The syndrome of transient headache and neurologic deficits with cerebrospinal fluid lymphocytosis is not extremely common but is listed in *The International Classification of Headache Disorders*. We reviewed clinical features of systemic conditions, laboratory tests, neuro-ophthalmological signs, the diagnosis, and treatment of this syndrome.

3. We reviewed drug-induced optic neuropathy caused by novel drugs, including targeted agents in cancer therapy, immunosuppressive agents, and biological agents.

4. Anti-aquaporin 4 antibody-positive optic neuritis, an intractable optic neuropathy, has recently been reported. We reproduced practical guidelines for this type of optic neuritis.

Ocular oncology and histopathology

1. Immunoglobulin (Ig) G4-related disease is a novel clinical entity characterized by infiltration of IgG4-immunopositive plasmacytes and an elevated concentration of serum IgG4 accompanied by various organs, including the lacrimal gland, salivary gland, and pancreas, that are enlarged and contain masses. Recent studies have clarified that conditions previously diagnosed as Mikulicz disease, as well as various types of lymphoplasmacytic infiltrative disorders of the ocular adnexa, are consistent with a diagnosis of IgG4-related disease. Against this background, the diagnostic criteria for IgG4-related ophthalmic disease have recently been established on the basis of the clinical and histopathologic features of the ocular lesions. This article reviews these new criteria with reference to the comprehensive diagnostic criteria for IgG4-related disease for all systemic conditions reported in 2012.

2. We reviewed the surgical approach for intraorbital tumors and stereotactic radiation therapy for optic nerve sheath meningioma.

Glaucoma

1. Analysis with the Markov model of the effects of a glaucoma examination program Glaucoma produces an irreversible visual field loss and is the main cause of visual

impairment in Japan. Early detection and treatment are important until the period of progression because symptoms are poor. We used the Markov model to analyze the effects of screening of adults for glaucoma. The early detection and early treatment of glaucoma are economically beneficial.

2. The purpose of treatment in patients with glaucoma is to maintain visual function and to reduce the intraocular pressure (IOP). We have used eyedrops as a medical treatment and usually pursue an operative treatment only when glaucoma is refractory to eyedrops. On the other hand, surgery for glaucoma changes the shape of the cornea, exacerbates astigmatism, and decreases visual acuity. Astigmatism can be divided into cases that can and cannot be corrected with lenses. Therefore, it is most important to examine what type of astigmatism is increasing. Recently, a device for analyzing the shape of the cornea was developed and has allowed detailed measurement of astigmatic quality. We are performing examinations with Orbscan (Bausch & Lomb Surgical, Rochester, NY, USA) and the OPD Scan corneal analyzer (Nidek Co., Ltd., Gamagori, Japan).

3. Because eyedrops are needed for the long-term treatment of glaucoma, patient compliance is important. For drug therapy, β -adrenergic receptor antagonist eyedrops have been used. Twice-daily administration was necessary, but several kinds of eyedrops that can decrease IOP for 24 hours with once-daily administration have recently become available. However, eye stimulation and foggy vision are a problem, because the eyedrops are a gel. However, when alginic acid is used as an agent, there are fewer side effects (stimulation and foggy vision); the same is true for long-acting carteolol hydrochloride eyedrops (Mikelan LA, Otsuka Pharmaceutical Co., Ltd., Tokyo). Therefore, we examined the effect of a change from twice-daily carteolol hydrochloride eyedrops to once-daily long-acting carteolol hydrochloride eyedrops on decreases in IOP and on their ease of use in patients with glaucoma. We found that the daily long-acting carteolol hydrochloride eyedrops improved compliance, were more convenient, and were equal to twice-daily eyedrops in lowering IOP.

4. Numerous studies have shown that human IOP in the sitting position is high in the morning and low in the afternoon and evening. When the subject is lying flat, IOP increases by as much as 2 to 6 mm Hg in both healthy persons and in patients with glaucoma. Recent data incorporating the concept of the habitual body position — sitting during waking hours and supine during sleeping hours — have demonstrated that peak IOP is most likely to occur at night while the patient is supine. The progression of visual field damage in normal-tension glaucoma is associated with IOP in the supine position and the magnitude of IOP elevation accompanying postural changes. It would be beneficial if treatment options were available that could specifically decrease the supine IOP, resulting in a reduction in the magnitude of IOP fluctuation caused by postural change. However, treatment with timolol maleate, latanoprost, or brinzolamide lowers IOP in both the sitting and supine positions but does not alter the response of IOP to postural change. The postural response is also reportedly unaffected by trabeculectomy without mitomycin C and argon laser trabeculoplasty. In patients with primary open-angle glaucoma or normaltension glaucoma, we evaluated the postural change in IOP following trabeculectomy with mitomycin C. The IOP was measured with a pneumatonometer after 5 minutes with the subject in the sitting position and after 10 minutes with the subject in the supine position. Sitting IOP and 10-minute supine IOP were 10.2 ± 3.3 mm Hg and 13.7 ± 4.5 mm Hg, respectively, and the difference between them (Δ IOP 10 min) was 3.43 ± 1.8 mm Hg (p < 0.05). Sitting IOP and Δ IOP 10 min were significantly correlated (r = 0.66, p < 0.0001). The lower the sitting IOP was, the lower \triangle IOP 10 min was.

Functional neuroimaging

Myelin is distributed in mainly the white matter of the brain. Yet in the gray matter, the myelin content depends on axonal projections respecting functional distribution. Some sensory, motor, and specifically associated cortices demonstrate higher myelin content than do adjacent cortical areas. Cortical myelination can be calculated with T1-weighted images divided from T2-weighted images as cortical myelin mapping with MRI. Detailed axonal projections to primary visual areas were examined with the cortical myelin mapping technique in patients with hemianopia who have altered optic radiation. Myelin mapping in these patients showed that myelin content was reduced in some areas, especially the posterior portion of the primary visual field defects. Cortical myelin mapping is also useful for estimating acquired axonal alteration reflecting detailed axonal projection to the cortex.

Developmental functional abnormality

Diffusion tensor imaging is a noninvasive technique to visualize axonal connectivity in the entire brain. Diffusion tensor images were acquired to evaluate axonal-axonal density by using fractional anisotropy on major white matter tracts to compare strabismic and control groups. The fractional anisotropy value of the strabismic group was reduced at the forceps major, which connects the occipital lobes via the splenium of the corpus callosum. The reduction of fractional anisotropy at the forceps major of the strabismic group suggests that some morphological alterations exist for the axons connecting both occipital lobes.

Visual neuropsychology

1. Assessment of plasticity and stability in the visual cortex and the visual pathway in patients with a lesion of either cones or retinal ganglion cells

We assessed the degrees of plasticity and stability of the visual cortex and the visual pathways in a patient who had a central scotoma with lesions of either cones or retinal ganglion cells. In addition to using fMRI as we have in previous studies, we performed diffusion MRI, which allows us to quantify the visual pathway, which consists of white matter, on the basis of the free movements of water molecules. Ogawa et al. have reported the results of quantification of the visual pathway in patients with a lesion of either cones or retinal ganglion cells.

2. Identification of cortical area for visual awareness

Because the human temporoparietal junction is a very large cortical region that responds to many kinds of stimuli, it has not been precisely mapped. Here, using fMRI and a mixture of visual and auditory stimuli, we revealed a small visually responsive area in the right temporoparietal junction, which has previously been suggested to play a role in visual awareness.

Low vision

On the basis of the results of our questionnaire survey (The comprehensive research for disabilities [sensory disability], H22-Sensory-general-005 by the Ministry of Health, Labour and Welfare), we created a software program, "First Step," and an Internet homepage, "Knowledge Bank," supporting persons with visual disabilities. We developed a new perimeter, "Active Field Analyzer," which can measure a visual search function that is a factor in the specificity of visual field, but not in the specificity of visual acuity, as revealed by a previous report (Practical verification of a next-generation supporting system for persons with visual impairment [sensory disability], H22-Sensory-general-005 by the Ministry of Health, Labour and Welfare).

Vitreoretinal diseases

We have used 23-, 25-, and 27-gauge transconjunctival vitrectomy system for macular hole, epiretinal membrane, macular edema and rhegmatogenous retinal detachment. The 25- and 23-gauge sutureless vitrectomy techniques decrease the surgical trauma and improve patients' postoperative comfort. The 25- and 23-gauge instrumentation is effective for a variety of vitreoretinal surgical indications. Although the infusion and aspiration rates of the 25- and 23-gauge instruments are lower than those for the 20-gauge high-speed vitrectomy system, the use of 25- and 23-gauge transconjunctival vitrectomy system may effectively reduce operative times of select cases that do not require the full capability of conventional vitrectomy.

To evaluate the clinical efficacy of the 7-mm IOL (Eternity[®], Santen Pharmaceutical Co. Ltd., Osaka, Japan) for combined pars plana vitrectomy, phacoemulsification, and IOL

implantation, we observed the visibility of the retina during vitrectomy and measured the depth of the anterior chamber preoperatively and postoperatively with the Pentacam[®] scanner (Oculus Optikgeräte GmbH, Wetzlar, Germany).

We are planning to evaluate the changes in regular and irregular corneal astigmatism after 25-gauge and 23-gauge transconjunctival sutureless vitrectomy.

We investigated changes in corneal thickness following vitreous surgery and determined whether such changes can be used as criteria for evaluating the invasiveness of vitrectomy.

To treat a lens nucleus that dropped during cataract surgery, we removed it through the corneal wound without using a pars plana vitrectomy.

Electrophysiology

We are recording electroretinograms (ERGs) to evaluate whether there are functional disorders at the retinal-cell level in hereditary retinopathy, retinal dystrophy, and macular disease. The ERG 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. In addition, we performed examinations with 4 kinds of recording system, such as the Ganzfeld stimulator, multifocal stimulation, color stimulation, and focal macular stimulation. In Ganzfeld stimulation, we recorded the responses separately from cone and rod cells of the retina according to international protocols. The multifocal stimulator, which reflects cone function, can record the responses separately from each element in 61 areas in the central 30 degrees around the posterior pole. Furthermore, stimulator results can be compared with visual field examination results and evaluated between subjective visual field examinations and objective ERGs. The color ERG records each response to separate long-, middle-, and short-wavelength cones. Recently, we acquired a focal macular stimulator. This stimulator can record the retinal function of the central 5, 10, and 15 degrees and is effective for searching for conditions, such as occult macular dystrophy, causing unidentified visual disturbance.

Diabetic Retinopathy section

We perform subtenon triamcinolone acetonide injection for diabetic macular edema at our outpatient clinic. After injection, decreases in macular retinal thickness are evident with optical coherence tomography (OCT), but macular edema recurs in some cases 3 months after injection. For cases of diabetic macular edema refractory to triamcinolone acetonide injection, we perform transconjunctival microincision vitrectomy with a 23-G trocar system. With this system, the scleral incision is small and can be closed without sutures. Other advantages are the decreased postoperative inflammation and surgical stress.

A group of vulnerable retina ganglion cells have been reported in patients with diabetes mellitus and in animal models of diabetes. We are recording ERGs to evaluate retinal function in patients with diabetes but without retinopathy, as shown with ophthalmoscopy. We measured the photopic negative response (PhNR) among wave patterns obtained with cone ERGs and examined the correlation between the PhNR and the duration of diabetes. We are measuring the thickness of the nerve fiber layer with OCT and are disordering the correlation of nerve fiber layer thickness with the PhNR amplitude or implicit

time or both.

Uveitis

1. We reported the first case of toxoplasmic central retinal artery occlusion and diffuse retinal vasculitis.

2. We reported a case of type 2 paracentral acute middle maculopathy.

3. We described detailed spectral domain optical coherence tomographic findings for 2 patients with focal choroidal excavation (FCE) associated with focal retinochoroiditis. The FCE can be seen as a tomographic phenotype after the treatment of focal retinochoroiditis. Spectral domain optical coherence tomography was useful for detecting the development of FCE after treatment and for observing the regression of FCE.

4. We reported a case of Behçet's disease with parafoveal macular ischemia associated with retinal vasculitis under low-dose oral contraceptives.

Macular degeneration

1. Dome-shaped macula was described by Gaucher and associates as a convex protrusion of macula within a staphyloma in highly myopic eyes which causes visual impairment associated with serous foveal detachment. We described a patient with spontaneous resolution of serous foveal detachment in a dome-shaped macula documented with serial spectral domain optical coherence tomography.

2. We compared the 3-year visual outcome after double therapy of photodynamic therapy (PDT) with intravitreal bevacizumab and the triple therapy of PDT combined with intravitreal bevacizumab and subtenon triamcinolone acetonide (STTA) injections for polypoidal choroidal vasculopathy (PCV). Initial therapy consisting of a single session of PDT combined with intravitreal bevacizumab and STTA improves vision in patients with treatment-naïve subfoveal PCV. Compared with double therapy, this triple therapy may be more effective for PCV.

3. We performed proteomic analysis to identify potentially specific biomarkers of exudative age-related macular degeneration. Prostaglandin H2 D-isomerase was identified in 10 cases. Because oxidative stress and chronic inflammation are involved in the development and progression of age-related macular degeneration, the identification of prostaglandin H2 D-isomerase is an intriguing finding.

Biochemistry

1. The therapeutic effects of cyclosporine A encapsulated in biocompatible and biodegradable blended nanoparticles of poly (lactic acid) homopolymers and polyethyleneglycol-block-poly (lactic acid) copolymers (stealth nanocyclosporine) were examined in an experimental autoimmune uveoretinitis model in Lewis rats. The strong therapeutic benefit on the model obtained with the stealth nanocyclosporine may have been due to prolonged blood circulation and targeting to the inflamed uvea and retina, in addition to sustained release in situ.

2. Many degenerative retinal diseases illustrate retinal inflammatory changes that include infiltration of microglia and macrophages into the subretinal space. We examined the role of chemokines in the Abca4(-/-)Rdh8(-/-) mouse model of Stargardt disease and

the Mertk(-/-) mouse model of retinitis pigmentosa. Our results indicated that chemokine (C-C motif) ligand 3 has an essential role in regulating the severity of retinal inflammation and degeneration in these mouse models.

Color vision defects and genetic analysis of retinal diseases

1. Retinitis pigmentosa and its allied disorders have genetic heterogeneity. In other words, there are many causative genes among these disorders. Although direct sequencing analysis for several causative genes is generally performed, there are few cases to identify causative gene mutations. Therefore, we have chosen whole-exome sequencing analysis to identify gene mutations. In fact, we have successfully identified several novel gene mutations.

2. We performed clinical and molecular genetic analysis of various inherited retinal diseases, such as retinitis pigmentosa and macular and conic dystrophies. We identified causative mutations in these diseases. To clarify disease haplotypes, the results of haplotype analysis with mutations were compared between family members and control subjects.

Cornea

The cornea group at The Jikei University chooses the ideal corneal surgery by discussing the various options with each patient.

Corneal transplantation has developed rapidly in recent years. Penetrating keratoplasty, a procedure consisting of full-thickness replacement of the cornea, has been the dominant procedure. Recently, lamellar transplantation surgery, which selectively replaces only diseased layers of the cornea, has becoming a standard procedure. A variety of corneal transplantation procedures with donor corneas can be performed according to the condition of the disease. We have performed Descemet's stripping automated endothelial keratoplasty for more than 30 patients and have obtained good postoperative results.

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Department of Otorhinolaryngology

Hiromi Kojima, Professor Atsushi Hatano, Associate Professor Yoshinori Matsuwaki, Assistant Professor Daiya Asaka, Assistant Professor Jiro limura, Assistant Professor Nobuyoshi Otori, Professor Makoto lida, Associate Professor Yoichi Seino, Assistant Professor Satoshi Chikazawa, Assistant Professor

General Summary

Our basic and clinical studies have examined: the pathogenesis of cholesteatoma, surgery for adhesive otitis media, image-guided surgery with intraoperative computed tomography scan update, space motion sickness, nasal allergy, endoscopic endonasal sinus surgery, endoscopic endonasal skull base surgery, sleep apnea syndrome, olfactory disorder, phonosurgery, deglutition, eosinophilic inflammation, and reconstructive surgery for head and neck tumors.

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. In regard to research on hearing loss, we are studying the physiology of the inner ear in metabolic disorders using experimental animal models and collaborating with Shinshu University in the genetic analysis of deaf patients.

We perform approximately 200 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 via the posterior cranial fossa approach, middle cranial fossa approach, or translabyrinthine approach, depending on the case.

For secretory otitis media we select the treatment method in individual patients depending on the degree of development of the mastoid air cells. With respect to the duration of placement of indwelling ventilatory tubes, we determine the timing of tube removal in each patient by measuring the changes in the middle ear total pressure caused by transmucosal gas exchange.

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. We also perform furosemide-loading VEMP as a test for patients suspected to have delayed endolymphatic hydrops. In addition, we are advancing research on the localization of the vestibular cortex and the projection from the vestibular system to the cerebral cortex by analyzing cerebral blood flow with single photon emission computed tomography in conjunction with the Department of Neurology.

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. Furthermore, intraoperative CT scan update for image-guided systems to adapt to anatomical changes during surgery is being developed. We have identified problems and possible areas of improvement relevant to this operative method and are altering the device to improve its accuracy and performance. 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 cadayers at the Skills Laboratory, for both skull-base surgery and endoscopic sinus surgery training. We must improve both medical techniques and anatomical knowledge. In addi-

tion, we started creating new methods of Internet access using telemedicine and a distance-training system. 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. In the future, we hope to perform clinical research on the expression of human papilloma virus, which has been implicated in the development of mesopharyngeal cancer and oral cancer, and to investigate treatments, such as vaccine therapy, for various cancers.

Research on vocal and swallowing functions

1. 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. To determine the optimal surgical indications and operative methods, we compare potential operative methods by means of fiberoptic laryngoscopy, stroboscopy, acoustic analysis, aerodynamic testing, and assessment using the Voice Handicap Index before and after surgery.

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.

2. 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. An important future task in this context is developing surgical treatment methods for patients who do not respond to botulinum toxin treatment.

3. Evaluation and treatment of dysphagia: We collaborate with other departments, such as the departments of neurology and rehabilitation, and include co-medical staff, such as nurses, in our treatment team. We consider therapeutic strategies by evaluating patients by means of video endoscopy and video fluorographic tests and are promoting swallowing training.

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. Therefore, we investigate the role of surgery, such as uvulopalatopharyngoplasty. 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.
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Department of Anesthesiology

Shoichi Uezono, Professor Naohito Shimoyama, Professor Masanori Takinami, Associate Professor Masaki Kitahara, Associate Professor Ichiro Kondo, Associate Professor Shigehiko Uchino, Associate Professor Kazuhiro Shoji, Assistant Professor Gumi Hidano, Assistant Professor Hiroshi Sunaga, Assistant Professor Sachiko Omi, Professor Shuya Kiyama, Professor Tsunehisa Tsubokawa, Associate Professor Chieko Fujiwara, Associate Professor Yasushi Mio, Associate Professor Yoshie Taniguchi, Assistant Professor Yoichi Kase, Assistant Professor Yukino Kubota, 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 2014 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 2014.

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, such as Grants-in-Aid for Scientific Research (*kakenhi*) and contract grants. The department continues to build on the strengths of several outstanding programs: cardio-vascular anesthesia, thoracic anesthesia, pediatric anesthesia, regional anesthesia, neuro-anesthesia, 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 2014 Dr. Tsubokawa and Dr. Fujii were recruited to renew cardiac anesthesia program with an emphasis on intraoperative transesophageal echocardiography. Their expertise on this technology will allow us to improve the quality of cardiac surgery, leading to better cardiac patient outcomes.

Our faculty and residents were both well represented at the Japanese Society of Anesthesiologists' annual meeting in Kobe and the American Society of Anesthesiologists' annual meeting in New Orleans. 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.

Doctor Uezono and his colleagues have been investigating the effects of neuromuscular blocking agents on the central nervous system. 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 will lead to therapeutic options being developed for this type of pain.

In clinical medicine, several principal investigators from the Department of Anesthesiology deserve mention. Doctor Kondoh 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, Dr. Kobayashi and his colleagues have been attempting to identify predictive factors affecting outcomes associated with artificial ventilatory therapy. 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.

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nary resuscitation in hypothermia-treated mice. Anesthesiology. 2014; **120:** 880-9.

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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 Hideki 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) traumatic brain injury, and 4) rehabilitation for children.

Research Activities

rTMS for stroke

1. Randomized, multicenter, comparative study of NEURO versus CIMT in poststroke patients with upper limb hemiparesis: the NEURO-VERIFY Study

The results of the 15-day rehabilitative protocol showed the superiority of NEURO (NovEl intervention Using Repetitive TMS and intensive Occupational therapy) relative to CIMT (Constraint-Induced Movement Therapy); NEURO improved the motion of the whole upper limb and resulted in functional improvement in activities of daily living

2. Brain perfusion and upper limb motor function: A pilot study of the correlation between evolution of asymmetry in cerebral blood flow and improvement in the Fugl-Meyer Assessment score after rTMS in chronic poststroke patients

Changes in the asymmetry index less than zero reflect improved perfusion, suggesting that the improvement of upper limb motor function in poststroke patients reflects the evolution of brain perfusion in the superior and middle frontal areas.

3. Continuous theta burst stimulation combined with intensive occupational therapy for upper limb hemiparesis after stroke: A preliminary study

The 15-day protocol of continuous theta burst stimulation combined with intensive occupational therapy is a safe and potentially useful method for treating upper limb hemiparesis after stroke.

4. Bilateral high- and low-frequency rTMS in patients with acute stroke and hemiparesis: A comparative study with unilateral high-frequency rTMS

Our proposed bilateral high- and low-frequency rTMS is safe and practical and showed a greater improvement of the Brunnstrom stage of the affected upper limb than did high-frequency rTMS. This novel rTMS approach may be a useful intervention for patients with acute stroke patients and hemiparesis.

5. Local injection of muscle with botulinum toxin type A synergistically improves the beneficial effects of NEURO in patients with spastic upper limb hemiparesis after stroke The triple-element protocol of rTMS, intensive occupational therapy, and local injection

of botulinum toxin type A into spastic finger muscles is a promising therapeutic program for spastic upper limb hemiparesis after stroke.

Dysphagia

1. Functional magnetic stimulation using a parabolic coil for dysphagia after stroke Functional magnetic stimulation with a parabolic coil can potentially improve swallowing function in patients with dysphagic after stroke.

2. Effect of early rehabilitation by physical therapists on the mortality of elderly inpatients after aspiration pneumonia

The data suggest that early rehabilitation by physical therapists was associated with a reduction in 30-day in-hospital mortality rates in elderly patients with severe aspiration pneumonia.

Traumatic brain injury

1. Characteristics of statistical imaging analysis in morphological and functional brain imaging of neuropsychological impairments and nonorganic mental disorders after traumatic head injury

Our results revealed several characteristics of statistical imaging analysis in functional and morphologic imaging of neuropsychological impairments and patients with nonorganic mental disorders after head injury.

Rehabilitation for children

1. Clinical efficacy of shoes and custom-made insoles in treating children with flatfoot The walking pattern of children with flatfoot can be changed with the use of shoes with custom-modeled insoles. Unlike barefoot children, children who wore such shoes achieved increases in both walking speed and step length and decreases in the swing phase and the walking angle on both sides.

Others

1. Effects of testosterone levels on functional recovery with rehabilitation in stroke patients

Our data suggest that serum level of free testosterone have a positive effect on the discharge functional independence measure in men who have had a stroke.

Publications

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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

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Department of Laboratory Medicine

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

General Summary

The members of our department performed studies about clinical laboratory medicine, with a focus on their individual specialties, as shown in the following *Research Activities*. Two research studies had started in clinical microbiology and clinical biochemistry with matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) from 2014. We also focused on insulin resistance, including nonalcoholic steatohepatitis (NASH). A simple method of using stable isotope breath tests to measure energy metabolism in the body will be developed in the near future.

Research Activities

Clinical microbiology

1. Identification of bacterial strains

Several clinically isolated, previously unidentified bacterial strains were identified though gene sequencing of polymerase chain reaction-amplified 16S ribosomal RNA. We investigated the relevance of the pathogen of *Staphylococcus aureus* and the phage open reading frame typing type. We have done research on the examination method of bacterial pathogens with MALDI-TOF MS.

2. Induction of the hepatitis B virus receptor Na⁺ taurocholic acid cotransporting polypeptide in the FLC-4 human hepatocellular carcinoma cell line by retinoic acid

The cell culture system that can reproduce the infection, replication, growth, and release of the hepatitis B virus (HBV) was the human hepatocytes culture system. The Na⁺ taurocholic acid cotransporting polypeptide (NTCP) on the cell membrane of hepatocytes was shown in 2012 to function as a HBV receptor. In the present study, we compared NTCP expression in several human hepatocellular carcinoma cell lines and examined the likelihood of establishing a new system for evaluating HBV infection. After comparing the NTCP messenger RNA expression in the FLC, Huh-7, and HepG2 cell lines, only FLC-4 cells significantly confirmed the onset of high NTCP, and the level of expression was higher than that by human hepatocytes but was 1/10 of that by differentiated HepaRG cells. Therefore, NTCP messenger RNA in FLC-4 cells was induced to a level similar to that of differentiated HepaRG cells with all trans-retinoic acid and the onset of the level of Na⁺-dependent. Thetaurocholic acid transportation activity was increased by approximately 20%.

Clinical chemistry

1. Mass analysis of urinary β 2 microglobulin in patients with proteinuria

We used using MALDI-TOF MS to analyze urinary β 2 microglobulin in patients with high proteinuria. Before mass analysis we purified urine samples to obtain β 2 microglobulin with the immunoprecipitation method. We detected the signal of 11.8-kDa intensity in the mass spectrograph, already reported as a reference. We also observed several different intensities in high molecular regions of the mass spectrograph. In patients with diabetes mellitus and proteinuria, we observed bimodal 11.8-kDa intensity. These results suggest that the part of the β 2 microglobulin of this research might be the polymerized form and might be modified by endogenous substance(s).

2. Measurement of lipoprotein cholesterol with high-performance liquid chromatography The vision of the high-performance liquid chromatography (HPLC) lipoprotein cholesterol measurement we developed was reported at a symposium of the 46_{th} annual scientific meeting of the Japan Atherosclerosis Society. The method to determine vitamin E concentrations of lipoprotein fractions developed with the modification of the HPLC method was published in Scientific Reports (2014; 4: 4086). In the studies of serum lipidrelated reagents, the accuracy and precision of each high-density lipoprotein cholesterol direct method was published in Atherosclerosis (2014: 233: 253-9) and the reference interval of apolipoprotein B48 was published in Journal of Atherosclerosis and Thrombosis (2014; 21: 618-27). The study to determine the high-density lipoprotein function of cholesterol efflux with stable isotope and the risk assessment of atherosclerosis received a Department of Education, Culture, Sports, Science and Technology Research Grant, and the fundamental method is now being developed. The collaborative study results of statin-related adverse events for glucose metabolism and renal function were published in Atherosclerosis (2015; 241: 409-18) and Public Library of Science One (2014; 9: e9691), respectively. The clinical significance of the eicosapentaenoic acid/arachidonic acid ratio to explain the atherosclerosis development determined by the cardio-ankle vascular index in patients with high serum concentrations of arachidonic acid presumably expressive of inflammation was published in International Journal of Cardiology (2014; 177: 517-9). 3. Difference of hepatic energy metabolism of male and female rats

Sex is an important factor for the onset of diabetes, liver cancer, and NASH. In women, impaired glucose tolerance and diabetes easily develop after menopause. The fasting ¹³C-glucose breath test has shown that the capacity of liver energy metabolism is higher in women than in men. Animal models are needed to clarify the causes of the sex gap in liver energy metabolism. The area under the curve until 6 hours of ¹³C excretion of female Wistar rats was higher than that of male Wistar rats. However, the difference in Long-Evans rats was opposite. The comparison of both types of rat will be useful for clarifying the sex difference in the mechanisms of liver energy metabolism. (Funded by the Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Strategic Research Foundation at Private Universities, 2011-2015)

Clinical pathology

Hepatic stellate cells that express lecithin:retinol acytransferase (LRAT) or cellular retinol binding protein 1 (CRBP-1) or both contribute to the development of fibrosis in patients

with NASH. The present study was performed to examine whether hepatic stellate cells (HSCs) positive for LRAT or CRBP-1 or both contribute to centrilobular fibrosis on NASH. Antibodies against LRAT and CRBP-1, a widely ascertained antibody to activated HSCs (α -smooth muscle actin [α -SMA]), and anti-R58 monoclonal antibody to latencyassociated protein of transforming growth factor- β degradation products (LAP-D) in cells or the matrix were used for immunohistochemical studies to assess the distribution of cells that contribute to the development of fibrosis. The HSCs positive for LRAT or CRBP-1 or both were stained in centrilobular lesions in fibrotic livers with NASH. These cells were observed in fibrotic septa, which were stained with α -SMA or LAP-D or both. The present study provides evidence that functional HSCs expressing LRAT or CRBP-1 or both and continuing to maintain the ability to store vitamin A contribute to the development of centrilobular fibrosis and parenchymal fibrosis in patients with NASH. (Funded by Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Strategic Research Foundation at Private Universities, 2011-2015) (In collaboration with the Departments of Internal Medicine and Pathology, The Jikei University School of Medicine)

Clinical psychiatry

We plan a clinical study of the management of pregnancy in woman with epilepsy. We discussed changes in serum concentrations of antiepileptic drugs during pregnancy. Furthermore, we reported a case of epilepsy that was induced by a specific situation and showed a peculiar clinical course. 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.

Addition

1. How to improve the certainty of laboratory communication

Because we work at the Department of Laboratory Medicine, transferring results to physicians correctly is important. However, we cannot confirm the receipt of the data. With oral transmission, the appropriate exchange and review of data are effective. Therefore, we investigate the frequency of review during daily work. To improve the accuracy of measurement and communication, we must pay more attention to the safety of transmission.

2. In the field of biochemical examination, we presented the effects of autoantibodies in tumor markers or hormone measurement, an examination of the SCC measurement method in an automated immunoassay analyzer (AIA-900, Tosoh Corp.), and the false-positive interference by human anti-mouse antibodies. We also announced in the field of microbiology a secular course on the sensitivity information for various antimicrobial agents in urine isolates and, in the physiological inspection area, we annouced a basic study of 10-m constant distance gait analysis with a walking spectrometer. With respect to laboratory management, we announced our approach for improving safety and shortening the inspection time.

Publications

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Department of Endoscopy

Hisao Tajiri, Professor Hiroo Imazu, Associate Professor Shoichi Saito, Assistant Professor Kenichi Goda, Assistant Professor Kazuki Sumiyama, Assistant Professor Tomohiro Kato, Professor Hiroshi Arakawa, Assistant Professor Keiichi Ikeda, Assistant Professor Hirobumi Toyoizumi, Assistant Professor

General Summary

Our main area of research is performing clinical studies of endoscopy for the diagnosis and treatment of gastrointestinal, 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), narrowband imaging (NBI), endocytoscopy, confocal laser endomicroscopy, endocytoscopy, and therapeutic endoscopy with a high degree of procedural freedom. Our published research outcomes and recent reports are summarized below.

Research Activities

Pharyngeal, esophageal, and gastric malignancies

1. Endoscopic diagnosis in esophagogastric neoplasia

Early detection and accurate diagnosis of premalignant and malignant lesions in the pharynx, esophagus, and stomach are essential to allow the most appropriate therapeutic strategy to be selected for each patient. In our research, we use several novel optical technologies, along with conventional white light endoscopy, in clinical cases. We have designed a series of prospective clinical studies to evaluate and validate these novel imaging technologies and their potential benefits. We introduced transnasal ultrathin endoscopy, which is expected to improve patient compliance. This type of endoscope reduces discomfort during examination, which makes it particularly useful for screening patients who are undergoing routine medical examination.

1) Magnifying endoscopic observation with an NBI system

This new diagnostic system consists of a magnifying (\times 90) endoscope and an NBI light source, which provides detailed morphological information about the capillaries on the mucosal surface. We studied the clinical utility of NBI-magnifying endoscopy for identifying superficial neoplasms in the pharynx, esophagus, stomach, and duodenum. In one study, we developed algorithms for NBI technology that would allow the histological type and tumor extent of gastric carcinoma to be determined without a biopsy. 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. In addition, we performed a single-center study to compare NBI-magnifying endoscopy with Lugol chromoendoscopy for detecting superficial carcinoma in the esophagus. Our goal is to evaluate this technology for the early detection of precancerous changes in the specialized columnar epithelium of Barrett's esophagus. The newly developed dual-focus endoscope and the updated NBI system have addressed the shortcomings of the conventional magnification scope and the NBI system. We prospectively compared dual-focus NBI with conventional magnification NBI for detecting superficial squamous neoplasms in the pharynx and esophagus. Results of these studies have been reported at several conferences and have been published in several English-language journals.

2) Endocytoscopy

Endocytoscopy is a novel optical imaging technique that allows the gastrointestinal mucosa to be visualized with a staining solution in vivo and in real time at the cellular level. We joined a multicenter study that employed endocytoscopy for the diagnosis of superficial esophageal squamous cell carcinoma. In addition, we performed a single-center study to determine the optimal staining regimen for in vivo endocytoscopy of normal mucosa and superficial neoplasms of the duodenum. We are now studying the characteristic endocytoscopy findings of superficial duodenal neoplasms, i.e., adenoma and mucosal adenocarcinoma. Furthermore, we are preparing manuscripts that describe the use of endocytoscopy for superficial esophageal neoplasms and superficial nonampullary duodenal epithelial tumors.

3) 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 it remains associated with a high false-positive rate, we have established that AFI, in combination with conventional white-light imaging and NBI, could improve specificity. This result was recently published in an English-language journal.

4) Ultrathin endoscopy (transnasal endoscopy)

Ultrathin endoscopy can reduce discomfort during endoscopic examination. However, ultrathin endoscopes have a poorer image resolution than do conventional endoscopes and, therefore, have a higher risk of false-negative results. Accordingly, we showed that ultrathin endoscopy was less able to detect gastric lesions than was high-resolution endoscopy. We are developing a method of studying esophageal motility disorders by using an ultrathin endoscope to assess symptoms evident during examination. Details of this motility study are described later.

5) Endoscopic ultrasound-guided fine-needle aspiration biopsy

Endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNA) allows histopathologic analysis of lesions that are typically undetectable with endoscopic examination. These lesions include those within the gastrointestinal walls, such as submucosal tumors of the esophagus and stomach, and mediastinal and lymph node lesions. In EUS-FNA, real-time ultrasonographic images are used for the precise guidance of the biopsy needle into lesions. Tissues obtained with EUS-FNA are immediately examined by a cytologist or pathologist to detect the presence of malignant cells. We are currently evaluating the technical safety and value of this technique in ongoing studies.

2. Endoscopic treatment of esophageal and gastric malignancies

Recent advances in endoscopic diagnostic techniques and instrumentation have led to the expansion of indications for endoscopic therapy in early gastric and esophageal carcinomas. Research on the following endoscopic therapeutic modalities is now underway to standardize their use for treating tumors of the upper gastrointestinal tract.

1) New indications for endoscopic treatment and endoscopic submucosal dissection Currently, we perform endoscopic submucosal dissection (ESD) for superficial neoplasms of the esophagus and stomach. En bloc resection with ESD is considered necessary to further develop endoscopic treatment. Successful development of a series of endoscopic knives and long-lasting submucosal fluid will reduce the technical difficulty of ESD and the risk of complications. We have monitored intragastric pH after endoscopy to evaluate the effectiveness of gastric acid-suppressing drugs, which have been used empirically after endoscopic treatment. We have also used blood cultures to study the risk of sepsis and endotoxemia after ESD.

2) Therapeutic interventions employing innovative endoscopy systems

The multibending scope (M-scope) is a new type of endoscope that provides greater access to sites that are typically difficult to reach. We have previously reported on the use of the M-scope in the treatment of tumors that are not accessible with conventional endoscopes: those of the lesser curvature, greater curvature, and posterior wall of the gastric body, and of the cardiac region. Furthermore, clinical studies using a newly developed therapeutic endoscope (the R-scope) are proceeding to advance the potential of endoscopic therapy. The, R-scope has a special mechanism allowing the forceps to move laterally and vertically, in addition to its multibending function. Because current endoscopic treatments are directed only at mucosal diseases but not transluminal diseases, we have also performed several studies using natural orifice translumenal endoscopic surgery, including full-thickness resection.

3. The role of Helicobacter pylori infection in the development of gastric cancer

The association between infection with *Helicobacter pylori* and the development of gastric cancer is well documented. However, many of the factors that affect this association are unknown. Determining these factors is important to our department because we routinely perform endoscopic treatment for gastric cancer. Experiments that address the association between infection with *H. pylori* and gastric cancer — particularly DNA methylation due to *H. pylori* infection — have been carried out in collaboration with the Department of Gastroenterology, Toshiba General Hospital. We have also been exploring the role of inducible nitric oxide synthase (iNOS) in the pathogenesis of *H. pylori*-associated diseases. We have demonstrated that the eradication of *H. pylori* plays an important role in the process of repairing disease-associated DNA methylation and in alterating the methylation patterns of genes in the mucosa in the 5 years after *H. pylori* has been eradicated. Interim results of this study have been reported at several conferences and have been published in Japan and internationally. In addition, we have reported that diverse topographical patterns of *H. pylori*-induced iNOS expression and iNOS gene polymorphisms may contribute to the development of gastric cancer.

4. Diagnosis of oropharyngeal and hypopharyngeal malignancies

Endoscopic screening with iodine staining, or Lugol chromoendoscopy, has allowed esophageal cancer to be detected at an early stage and has thus improved prognoses.

However, this technique is difficult to perform in certain locations, such as the oropharynx or hypopharynx. Metachronous or synchronous cancer of the oropharynx or hypopharynx is the main factor impairing the prognosis and quality of life of patients with esophageal cancer. 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. We performed a multicenter randomized controlled study of the clinical value of this new combination endoscopy. In addition, we performed a single-center study to evaluate the endoscopic characteristics of superficial carcinoma in the pharyngeal region. These results have been reported at medical congresses and in English-language medical journals.

Functional disorders of the upper gastrointestinal tract

The causes of gastroesophageal reflux diseases, including nonerosive reflux disease and gastrointestinal motility disorders, are difficult to determine. Establishing methods to evaluate the hypersensitivity and dysmotility of the gastrointestinal tract are important for understanding disease pathophysiology and for selecting effective treatment. Hence, we have developed a new method of evaluating esophageal functions with a small-caliber endoscope. We have started basic experiments on esophageal motility and sensitivity, with the goal of transforming this technique from a research tool into a clinical tool.

Diagnosis and treatment of esophagogastric varices

We have recently been involved in color-Doppler endoscopic ultrasonographic studies of the hemodynamics of the portal venous system in patients with esophagogastric varices. These studies have identified several factors that increase the likelihood of esophagogastric varices recurring after endoscopic treatment. When all such factors have been identified, we will be able to predict and prevent early recurrence of varices after treatment. We have also started a study to confirm the factors that exacerbate hemorrhagic gastritis and cardiac varices. Color-Doppler endoscopic ultrasonography is also highly accurate for detecting gastrorenal shunts, which can complicate the treatment of esophagogastric varices, and can delineate shunts in detail. Therefore, this diagnostic system will be useful for selecting patients with esophagogastric varices who are candidates for treatment with interventional radiology and for predicting the efficacy of treatment.

Enteroscopy and colonoscopy

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. Capsule endoscopy was performed in more than 1 million cases worldwide before May 2011 and is highly recommended as a first-line examination to detect disease of the small intestine. However, because capsule endoscopy is purely diagnostic, we have introduced singleballoon enteroscopy, which allows biopsy and hemostasis to be performed for hemorrhagic lesions of the small intestine.

The number of cases of colonic cancer in Japan has increased markedly. In major Euro-

pean countries, many studies have described the use of capsule endoscopy for examining the colon. In Japan, we are collaborating with another facility to perform studies with capsule endoscopy to screen patients for colonic neoplasms.

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. Hence, to maximize our diagnostic accuracy, we use a magnifying endoscope with NBI and crystal-violet staining, AFI technology, or both, along with conventional white-light observation.

2. Research in endoscopic interventions

Surgical resection has been the treatment of first choice for large, flat, elevated tumors of the colon. Recently, endoscopic en bloc resection performed with ESD (a standard treatment for gastric lesions) has been used for such colonic lesions. However, the endoscopic resection of large intestinal lesions is technically difficult because of the large intestinal folds and the higher rate of complications, such as perforation and bleeding. We are currently working to establish safe and reliable endoscopic methods for the complete removal of large colonic lesions to start preliminary use of ESD. In addition, we have used an infrared endoscopy system to evaluate the risk of bleeding from vessels located at the base of ESD-induced ulcers.

3. Capsule endoscopy and enteroscopy

Capsule endoscopy is a minimally invasive endoscopic modality that can be used to detect lesions of the small intestine which are inaccessible with traditional push-type enteroscopy. Recently, particularly in Western countries, capsule endoscopy has been recommended as the first-line endoscopic examination for evaluating and managing obscure gastrointestinal bleeding. We have performed capsule endoscopy for 850 patients, since the Japanese health insurance system began covering this procedure in April 2007. We determined that capsule endoscopy should be performed immediately after a patient presents at the hospital with a complaint of melena. Our goal is to continue to improve the diagnostic accuracy of capsule endoscopy for evaluating obscure gastrointestinal bleeding by re-evaluating the traditional bowel preparation regimen.

4. Basic research

Hyperplastic polyp is defined as a nonneoplastic tumor of the colon. Therefore, endoscopic treatment is not indicated on a histologic basis. However, according to recent reports from Western countries, sessile serrated lesions (SSA) can develop into advanced, invasive submucosal cancers that invade deeper layers of the submucosa. We are presently studying the use of the AFI and NBI systems to detect neoplastic lesions, including sessile serrated polyps (SSA/P). We are also examining biological markers of malignancy in SSA/P, by means of immunohistochemical staining, to evaluate whether such lesions have malignant potential.

In contrast, submucosal invasive cancer, which invades into a depth of less than 1,000 μ m, is an indication for endoscopic treatment, according to the 2014 Guideline for the Treatment of Colorectal Cancer from the Japanese Society for Cancer of the Colon and Rectum. Therefore, such invasive cancer warrants further investigation with magnifying endoscopy. We are currently studying the characteristic findings of conventional and magnifying endoscopy. In cases in which the submucosal invasion depth is the only compo-

nent that does not satisfy the criteria for a radical-cure evaluation, and no other risk factors for metastasis are observed, the rate of metastasis to lymph nodes has been reported to be extremely low. Our university is included in an ongoing investigation of the stratification of risk factors for the metastasis of deep submucosal invasive cancers (invasion depths >1,000 μ m) to other organs.

Pancreatobiliary endoscopy

1. Diagnosis of biliary and pancreatic diseases

Because of the recent introduction of the Diagnosis Procedure Combination (a specialized Japanese insurance system), establishment of a standardized, systematic diagnostic algorithm for biliary and pancreatic diseases has become more important than ever. We are comparing the diagnostic accuracy of EUS-FNA, multidetector-row computed tomography, magnetic resonance cholangiopancreatography, and endoscopic retrograde cholangiopancreatography (ERCP) in hepatopancreatic diseases. We have also introduced second-generation contrast media for ultrasonic imaging in the EUS diagnosis of 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-FNA. Moreover, we are developing a novel molecular imaging system using EUS with microbubbles for pancreatic carcinoma.

The technique of ERCP is well established, but it is associated with a risk of severe complications. To help address this problem we designed a new catheter and multibending duodenoscope to reduce unplanned pancreatic injection of contrast medium, which is considered a major cause of the common complication of post-ERCP pancreatitis.

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.

Palliative care

Palliative care has gained increasing interest. Various techniques have been developed to provide the best quality of life for critically ill or terminally ill patients. Endoscopic procedures may play an important role in palliative care, especially in supporting food intake. In our department, percutaneous endoscopic gastrostomy is performed for patients who are unable to maintain sufficient oral intake of food. Although percutaneous endoscopic enterostomy is not conventionally indicated for patients who have undergone gastric surgery, since 1994 we have extended the use of this procedure to include such patients and have investigated the clinical usefulness of the technique. We have developed kits for percutaneous endoscopic enterostomy placement. To alleviate stenosis caused by tumors of the digestive tract and bile duct, we have performed endoscopic bal-

looning/bougienage and subsequent metallic stenting and have obtained good therapeutic results. To reduce the pain associated with chronic pancreatitis and inoperable pancreatic cancer, we have performed transgastric celiac plexus blocks using EUS. These endoscopic procedures may greatly improve the quality of life of patients who are not candidates for radical surgery. The cost-effectiveness of these interventions is also beneficial.

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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 Summary

Rapid diagnosis and appropriate antimicrobial treatment are required for a good prognosis in cases of bacterial infection. Because an antibiotic must be administered as an empiric therapy before a pathogen has been detected, we should know bacterial epidemiology and antibiograms. Our investigations have shown that extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* should be considered, even for a communityacquired bacteremia. In addition, we have shown that methicillin-resistant *Staphylococcus aureus* (MRSA) derived from bacteremia in our hospital was susceptible to vancomycin, teicoplanin, linezolid, daptomycin, and arbekacin. Although both studies included small numbers of patients, they demonstrated important results.

To prevent the spread of pathogens, all medical staff should understand and carry out infection control. The "cross monitoring and feedback" is an extremely important tool for these practices. In addition, the infection control team (ICT) will promote an appropriate antimicrobial treatment and may prevent emerging antibiotic resistance.

Research Activities

Clinical studies of patients with bacteremia due to ESBL-producing E. coli

We investigated the clinical features and outcome of 19 patients with bacteremia due to ESBL-producing *E. coli*. The patients' median age was 61 years. Sixteen (84.2%) of the 19 patients had a community-acquired infection, and 15 (93.8%) of these 16 patients had been admitted from a nursing home or other hospital. The source of infection was the urogenital tract in 13 patients (68.4%). Severe sepsis or septic shock was present in 6 patients (31.6%). The efficacy rate of initial treatment was 100% with a carbapenem (meropenem, doripenem), 85.7% with tazobactam/piperacillin, 75.0% with cefmetazole, and 0% with other antimicrobials. Crude mortality was 10.5%. All patients who died had been treated with other antimicrobials. These results indicate that the most adequate agents of empiric therapy for bacteremia due to ESBL-producing *E. coli* are carbapenems. Further study is needed of the efficacy of tazobactam/piperacillin and cefmetazole.

Nontechnical skill has been successfully applied to hospital infection control

We have attempted to educate healthcare workers by means of TeamSTEPPS (Team Strategies and Tools to Enhance Performance and Patient Safety), which is one of the most popular educational tools for nontechnical skills. The "cross monitoring and feedback" tool was applied to develop the hand hygiene compliance in each hospital unit. After these efforts, the total amount of alcohol hand rub consumption was 23 ml/patient-days (ptd) in 2013 and 32 ml/ptd in 2014. The incidence index of newly nosocomial MRSA carriers was 0.21/1000 ptd in 2013 and 0.12/1000 ptd in 2014. The TeamSTEPPS is useful for developing infection control practice in our hospital.

Human immunodeficiency virus testing: Relationship between reasons for testing and late diagnosis

We attempted to clarify the rate of late diagnosis of human immunodeficiency virus (HIV) infection and identify the relationship between the reasons for HIV testing and a late diagnosis. Our retrospective cohort study among HIV-positive patients at The Jikei University Hospital was performed from 2001 through 2014. Patient characteristics from medical records, including age, sex, sexuality, reason for HIV testing, and the number of CD4-positive lymphocytes, when HIV was diagnosed were assessed. A total of 459 patients (men, n = 437; 95.2%) were included in this study, and their median age at HIV diagnosis was 36 years (range, 18-84 years). The rate of late diagnosis (CD4 cell counts $< 350/\text{mm}^3$) was 61.4% (282 of 459 patients), and the rate of very late diagnosis (CD4 cell counts $< 200/\text{mm}^3$) was 36.6% (168 of 459 patients). The most common reason for HIV diagnosis was voluntary testing (38.6%, 177 of 459 patients) and was followed by acquired immunodeficiency syndrome (AIDS)-defining illness (18.3%, 84 of 459 patients). Multivariate analysis revealed a significant association of voluntary HIV testing with non-late and non-very late diagnoses and also revealed that the late and very late diagnosis groups had higher proportions of AIDS-defining illness than did other groups. MSM (Men who have sex with men) was a factor for non-late diagnosis, whereas nonspecific abnormal results of blood tests, such a hypergammaglobulinemia and thrombocytopenia, were risk factors for very late diagnosis. Voluntary HIV testing should be encouraged, and physicians should screen all patients who have signs or symptoms, particularly hypergammaglobulinemia and thrombocytopenia, which may nonspecifically indicate HIV infection.

Infection Control Team Ward Rounds for Gram-negative bacilli bacteremia

We analyzed the Infection Control Team ward rounds (ICT round) result for Gram-negative bacilli bacteremia. The 36 cases (23 in men and 13 in women) of Gram-negative bacilli were detected in blood collected in March through May 2015, the median age of patients was 71.6 years, and 18 of the cases were in patients with malignancy. Of the 36 cases, 22 were of community onset and 14 cases were of hospital onset. The portals of entry were the biliary tract in 16 cases (44.4%), the gastrointestinal tract in 11 cases (30.6%), and the urinary tract in 8 cases (22.2%). The most common bacteria, including 3 cases in which 2 species had been detected at the same time, were *E. coli* (50%, 19 cases) and *Klebsiella pneumoniae* (32%, 12 cases). Initial antibiotics were sulbactam/cefoperazone in 12 cases (33.3%), tazobactam/piperacillin in 6 cases (16.7%), and meropenem in 5 cases (13.9%). According to susceptibility, the appropriate antimicrobial agent was changed in 15 cases (42%, including 2 cases of escalation against ESBL) and de-escalation was not properly performed in 8 cases (22%). Deaths within 30 days occurred in 2 cases. Antimicrobial stewardship can be promoted by the ICT round for Gram-negative bacilli bacteremia. More accurate ICT intervention is a future challenge.

Drug resistance in HIV infection

The recent evolution of antiretroviral agents, such as those with more activity, higher genetic barrier, fewer side effects, and less drug burdens, has achieved highly successful rates of HIV treatment. However, the resistance of HIV to drugs is an occasional problem. Drug-resistant HIV has several causes, but the most important factor is patient adherence. The treatment for drug-resistant HIV infection requires active antiretroviral agents and greater patient adherence. Furthermore, it is necessary to consider drug-drug interaction and side effects of antiviral agents that restrict the treatment options in clinical practice. Because drug-resistant HIV infection has no typical management, accurate consideration is needed for each case.

Surgical site infection due to Clostridium difficile

The bacterium *Clostridium difficile* colonizes healthy persons as a standard bacterial flora of the intestinal tract but can induce colitis after an antibiotic has been used. However, extraintestinal manifestation due to *C. difficile* is rare. A 71-year-old woman was hospitalized for suturation of the left Achilles tendon, which had ruptured because of an accident. The antibiotic cefmetazole was administered for 4 days during the perioperative period. After 2 weeks, the surgical site was opened with effusion, and *C. difficile* was isolated from the culture of this site. The *C. difficile* was not isolated from the patient's diarrhea or stool, and *C. difficile*-related colitis was not diagnosed in any other patients of the ward. Therefore, we could not identify an earlier patient with *C. difficile* infection. Thus, physicians should be more aware of extracolonic manifestations of *C. difficile*. Furthermore, we will investigate the risk factors for extracolonic manifestations of *C. difficile*.

Analysis of pathogenic factors of MRSA isolated from 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 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. To analyze the predictive factors for metastatic infection due to MRSA bacteremia, we performed a broth microdilution method for the antibiotic susceptibility testing of 10 isolates that were clinically isolated during 2013. All 10 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.

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Department of Dentistry

Katsuhiko Hayashi, Professor Shigeru Suzuki, Assistant Professor Akihiro Ikai, Professor

General Summary

1. linical studies of temporomandibular disorders

We continued our studies of screening questionnaires and evaluation of quality of life in patients with temporomandibular disorders (TMDs).

2. Anatomical studies of the temporomandibular joint of marsupials

We continued our anatomical and histological examinations of the temporomandibular joint (TMJ) and articular disk in Mammalia.

3. Clinical studies of obstructive sleep apnea/hypopnea syndrome

We studied evidence of fatty metamorphosis in the lingual muscles using computed tomographic (CT) images of patients with suspected obstructive sleep apnea/hypopnea syndrome (OSA/HS). We compared CT with cephalometric radiography for evaluating measurements of OSA/HS and report that we examined the difference.

4. Clinical studies of perioperative oral management

We reported the present situation of perioperative oral management and also investigated the effects of perioperative oral management on the length of intensive care unit (ICU) stay and the length of the postoperative hospital stay.

5. Clinical studies of occlusal discomfort

We gathered data and evaluated the conditions associated with patients with occlusal discomfort at multiple institutions.

Research Activities

Clinical studies of TMDs

1. Examination of personal computer operating time and clinical background factors of patients with TMDs at general clinical offices in metropolitan Tokyo

We developed a test for screening for TMDs and reported on the relationship between the development of TMDs and personal computer (PC) operating time with the cooperation of the Tokyo Dental Association in 2011. In the present study, we examined the differences in the relationships of PC operating time and the TMD prevalence between 2 groups. We classified the subjects into 2 groups: a 2007 + 2009 group whose data were previously reported and a 2012 + 2013 group whose data were prevented. The PC operating time was higher in the 2012 + 2013 group, but the TMD prevalence rate did not differ significantly between the groups. The mean age did not differ significantly between the 2007 + 2009 group (34.3 years) and the 2012 + 2013 group (35.3 years). The mean PC operating time was significantly greater in the 2012 + 2013 group (7.3 hours) than in the 2012 + 2013 group (4.2 hours). On the other hand, the groups did not differ significantly in sleeping hours, hours until sleeping after coming home, or commute time

extended. The association of prevalence rates of TMDs and sleeping hours, PC operating time were no statistically significant; therefore, we suspect that guidelines for visual display terminal work were observed and that the sleep situation might have been improved. We believe that investigates should include the PC environment, namely visual display terminal work environment, and the sleep situation.

2. Evaluation of therapeutic effects using the limitation of daily functions questionnaire in patients with TMDs

This study evaluated the pain-related limitations in daily functions before and after treatment in patients with TMDs by using the limitations in daily functions TMDs questionnaire (LDF-TMDQ). The relationship between improvements in LDF-TMDQ scores and improvements in other variables was analyzed by means of structural equation modeling. The LDF-TMDQ is suitable for evaluating the therapeutic effects of TMDs because changes in the scores were proportional to therapy-induced improvements in mouthopening range, pain intensity, and chewing difficulty.

Anatomical studies of TMJ of marsupials

1. Absence of the articular disk in the monotreme TMJ

This study investigated the morphological characteristics of the TMJ in monotremes through anatomical and radiological examinations. We reconfirmed the absence of the articular disk in the TMJ of the platypus and echidna after a unique report in 1900. Current anatomical and radiological findings indicate that mastication occurs by a combination of lateral and vertical jaw movements in the platypus and involuntary vertical jaw movement accompanied by thrusting of the tongue in the echidna.

Clinical studies of OSA/HA

1. The influence of fatty changes in lingual muscles and body mass index on the apnea hypopnea index

It has recently been postulated that a change in muscle function is associated with the etiology of OSA. Saito and colleagues have previously reported the effect of obesity on the properties of the lingual muscles (genioglossus and geniohyoid) in rats (Arch Oral Biol (2010; 55(10): 803–808). However, based on previous images, fat to muscle metamorphosis has not been shown in humans. Here, we show evidence of fatty metamorphosis in the lingual muscles using CT images of patients with suspected OSA. The subjects were 62 patients (47 men, 15 women) with suspected OSA who visited the Tsurumi University School of Dental Medicine from November 2007 through October 2011. Subjects underwent CT evaluations at the image diagnosis department of the hospital. Sex, age, body mass index (BMI), and apnea hypopnea index (AHI) were recorded for each patient. Inferior airway space and the total value of length and width of the inferior airway space (TIAS) were also measured. The degree of fat-to-muscle metamorphosis was measured with CT. Values were quantified and compared statistically.

Consistent with the report of Saito et al, we showed evidence of fatty metamorphosis of lingual muscles of humans with effects of the TIAS and AHI.

2. Examination about the evaluation of maxillofacial form and soft tissue form in sleep apnea syndrome

Cephalometric analysis has been said to be more effective for evaluating sleep apnea syndrome. However, cephalometric analysis insufficiently evaluates soft tissue. On the other hand, CT is performed with the patient in the supine position. Some reports have compared CT with cephalometric radiography, but the evaluations, such as detection powers, are not accomplished. We inspected sample size and detection power afterwards. We compared CT with cephalometric radiography regarding the evaluation measurements of sleep apnea syndrome.

The detection power was 1.0 at sample size 385 (Gpower). Bone form measurements differed significantly between cephalometric radiography and CT. However, we judged no clinical difference because the difference was all less than 1 degree. In the soft-tissue form, CT measurements (distance from the mandibular plane to the hypoid bone, posterior airway space, and distance between the posterior nasal spine and the tip of the soft palate) significantly showed small values. The measurements in cephalometric radiography and CT did not have a clinical difference in the bone form, and there was an important clinical difference in the soft-tissue form.

Clinical studies of perioperative oral management

1. Current and future status of perioperative oral management

Perioperative oral management was announced by revision of the medical treatment fees in 2012, and we care for patients actively. We performed research to clarify the situation of perioperative oral management.

The subjects were patients who had undergone organ transplants, marrow transplants, cardiac surgeries, chemotherapies, radiation therapies, or malignant surgeries of the head and neck, respiratory organs, or digestive organs under general anesthesia. We created an anonymous database to examine dental treatment regimens, departments that request patients, and periods between the first dental examination and the first treatment of primary diseases. We then compared the results in the first year with those in the second year. The number of patients who require perioperative oral management has been steadily increasing, and patients from the departments of otorhinolaryngology, hematology, oncology, and cardiac surgery account for 80% of the all patients. The problems are not enough for time of dental treatment. Patients usually do not understand the importance of oral healthcare before their treatment and operation. We introduce the importance of perioperative oral management to medical departments and nurses in our hospital.

2. The cooperation with cardiac surgery in our hospital under perioperative oral management

We have attempted to facilitate cooperation between medicine and dentistry by using a schedule we developed of medical examination incorporated with dental treatment during a preoperative examination, a request form for dental treatment and other fields, and a pamphlet recommending dental treatment during a preoperative period. In the prevent study, we reported the present situation of perioperative oral management in cases of cardiac surgical procedures and also investigated the effects of perioperative oral management on the lengths of ICU stays and postoperative hospital stays. Although no significant difference in the length of ICU stays was found, the results showed a significant difference in the length of postoperative hospital stays.

Comprehensive dental care support during the preoperative period in perioperative oral management is believed to help shorten the postoperative hospital stay; our study had a similar result. To provide enough preoperative oral care, cooperation should be facilitated between medicine and dentistry and early dental intervention should be encouraged. Future studies will need to examine the effectiveness of dental care support based on detailed clinical assessments of patients' background factors, such as complications and surgical methods.

Clinical studies of occlusal discomfort

1. A multi-institution investigation of the status of patients who complain of occlusal discomfort

We gathered data and evaluated the conditions associated with patients who have occlusal discomfort at multiple institutions. The patients were 143 women and 37 men with a median age of 55.0 years. The most common chief complaint was contact of the intercuspal position. Although the site of occlusal discomfort was often the molars, the site could also be the incisors. Additionally, occlusal discomfort was experienced in various locations, including one side or both sides, and many patients felt discomfort in the full dental arch. Although occlusal discomfort was often caused by metallic prostheses, such discomfort was also present in natural teeth. The most common treatment was previously prosthetic dental care. Although the sex and median age of patients were the same as those in previous reports, the duration of illness varied. The patients experienced occlusal discomfort in treated teeth and also in natural teeth in various locations.

Publications

Nishiyama A¹, Kuruma E, Hayashi K, Tsukagoshi K¹, Kino K¹, Sugisaki M (¹Tokyo Med Dent Univ). Evaluation of therapeutic effects using the limitation of daily functions questionnaire in patients with temporomandibular disorders. *Oral Health Dent Manag.* 2014; **13:** 982-6.

Department of Transfusion Medicine

Tetsunori Tasaki, Professor

Yoko Kato, Associate Professor

General Summary

1. Safe blood transfusions depend on lowering the risks from allogeneic blood units, which might be contaminated by infectious agents or inflammatory cytokines that have accumulated during storage, and on the appropriate use of transfusions. Educating medical and nursing students and practicing physicians about transfusion medicine is therefore extremely important for improving the safety of blood transfusions. Education includes the requirement that students perform transfusion testing. The tests they learn to perform include ABO typing, screening for irregular antibodies, and cross matching. They are also required to learn more about the blood program at the regional blood center.

2. Among the adverse reactions to blood transfusion, dyspnea has now become of greatest concern worldwide. Well-known causes of this adverse reaction include transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), anaphylaxis, and transfusion of ABO-incompatible blood. Differentiating between TRALI and TACO is extremely important, because the treatment of each condition is quite different, although affected patients present with similar signs and symptoms. Therefore, clinically significant and useful diagnostic criteria are needed.

3. Diagnostic criteria for TRALI were established in 2004 at the consensus conference held in Toronto. However, the criteria did not refer to antileukocyte antibodies in the donor blood transfused to the patient, although the development of TRALI and dyspnea appears to be associated with antileukocyte antibodies, and the evaluation of antileukocyte antibodies for the diagnosis of TRALI is now being considered.

4. Various adverse reactions, such as nausea, vomiting, and fever, are observed after hematopoietic stem cell transplantation (HSCT), because of the simultaneous transfusion of an anticoagulant (acid-citrate-dextrose, heparin) and a cryoprotectant (dimethylsulfoxide). Detailed knowledge of the timing, types, and frequency of specific signs and symptoms associated with these reactions may be useful for developing preventive therapy.

Research Activities

1. Additional education on transfusion reactions includes teaching the residents about the appropriate management of transfusion-associated adverse reactions. Although the Japanese Red Cross Society and the Japan Society of Transfusion Medicine and Cell Therapy (JSTMCT) have been collecting information on adverse reactions, they cover too much information and appear to be inadequate educational resources. Fujii, Tasaki, and colleagues have created a practical guide for the management of transfusion reactions. This PDF guide was written in both English and Japanese and can be seen at the Website of the JSTMCT (http://yuketsu.jstmct.or.jp/). The significance of the guide in the education of transfusion medicine was reported at the International Society of Blood Transfusion meet-

ing held in Seoul, South Korea (Fujii Y et al. Vox Sang 107, 2014, 29-30).

2. To establish useful guidelines for the diagnosis of TACO, Tasaki, as the principal investigator, formed a study group with his colleagues in 2013, using a Health and Labour Science Research Grant. The new proposed definition of TACO is composed of 6 primary features and 5 items to support diagnosis. Twenty patients in whom TACO was diagnosed with guidelines proposed by the Japanese Red Cross were reinvestigated with the use of our new guidelines. Seven of 20 patients were diagnosed as transfusion associated dyspnea other than TACO, primarily because there was not enough of the type of data required by our guidelines to perform an accurate diagnosis. However, the results also indicated that proper evaluation of the patient's clinical signs and symptoms by a physician was the most important factor for the final diagnosis, although detailed laboratory data would be desirable.

3. The relationship between antileukocyte antibodies in donor blood and dyspnea in the recipients was investigated prospectively, because these antibodies are found in almost 30% of patients in whom TRALI is diagnosed. A total of 601 specimens from female donors of platelets were screened for antileukocyte antibodies. Seventy of 601 specimens (11.6%) were positive for the antibodies, and 24 of the 70 patients receiving the positive units were investigated for adverse reactions by examining their medical records. However, there was no significant relationship between the development of adverse reactions and the receipt of positive units. From 14 patients whose HLA typing had been completed, specific information on the antibodies in the platelet unit they had been given was identified with LABScreen Single Antigen assays. At least 1 locus of HLA was identical to the specificity of the antibodies in 3 patients, but no positive association with adverse reactions was seen. Because the occurrence of TRALI is rare, prospective validation of a positive association between antileukocyte antibodies in transfused blood products and the development of adverse reactions, such as dyspnea, seems difficult to perform by studying a small number of cases. These results were reported at the AABB meeting in Philadelphia (Tasaki T et al. Transfusion 54 (suppl), 2014).

4. A new study group was formed by Ohto et al. (Fukushima Medical University) to identify the adverse reactions associated with HSCT. As of May 2015, of 32 patients receiving HSCT, 18 (56.3%) had 1 or more adverse reactions, such as elevated blood pressure (9 patients), hematuria (12 patients), and fever (2 patients). However, to date, there have not been any serious reactions requiring emergent therapy.

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

Ubiquitin proteasome and autophagy-lysosome systems in unloading induced skeletal muscle atrophy

Ubiquitin proteasome systems are considered to act in coordination with autophagy-lysosome systems. We investigated the involvement of neural precursor cell expressed developmentally down-regulated protein 4 (Nedd4) and autophagy-related proteins in unloading induced muscle atrophy with a specific focus on age dependency. Unloading induced higher expression of ubiquitin ligase Nedd4 and autophagy-related proteins, especially in aged rats, independently of intermitted reloading sufficient for preventing atrophy. Reloading may have induced an anabolic process that overwhelms the catabolic process of the ubiquitin-autophagy system.

Site-specific effects of nutrition and exercise on rat musculoskeletal system

Both nutrition and exercise are necessary to maintain musculoskeletal function. We studied the site-specific effects of nutrition and exercise on the musculoskeletal systems of growing rats. Dietary restriction suppressed exercise-induced down-regulation of myostatin with a corresponding increase in muscle mass in the plantaris muscle but not in the soleus muscle. Exercise selectively increased the bone volume and mineral density of trabecula in metaphyses. This increase was suppressed by dietary restriction. We concluded that dietary restriction suppresses the exercise-induced growth of the musculoskeletal system differentially in a site-specific manner.

Effects of daily exercise with dietary restriction on fatty liver and skeletal muscle morphology in Zucker fatty rats

Daily exercise and dietary restriction have been strategies for improving obesity-related diseases. We studied the effects of daily exercise or dietary restriction or both on fatty liver and the morphology of skeletal muscle in genetically obese Zucker rats. Daily exercise combined with dietary restriction, but not dietary restriction alone, improved insulin resistance, hyperlipidemia, fatty liver, and hepatic fatty acid oxidation with higher expression of carnitine palmitoyl-coenzyme A transferase 1. In addition, daily exercise combined with dietary restriction attenuated lipid accumulation and mitochondrial swelling within myofibers. These findings correlated with improvements in insulin resistance and

may reflect the amelioration of lipid metabolism.

Astaxanthin supplementation effects on adipocyte size and lipid profile in Otsuka Long Evans Tokushima Fatty rats

We investigated the effects of astaxanthin supplementation (2 g/kg diet powder chow food) on visceral adipose tissue and the lipid profile in Otsuka Long Evans Tokushima Fatty rats, an animal model of hyperphagia and visceral fat accumulation. Astaxanthin supplementation for 6 weeks promoted the size reduction of visceral adipocytes, decreased free fatty acid levels, and increased the serum level of high-density lipoprotein cholesterol. These results suggest that astaxanthin can at least partially ameliorate the obesity-related lipid profile and adipocyte abnormalities.

Clinical definition and diagnostic criteria for sarcopenia

The occurrence of sarcopenia and muscular dystrophy with aging has been attracting more attention. Many factors are reported to cause sarcopenia, such as the functional decline of a digestive organ occurring with aging and malnutrition due to a decrease in food intake. Other reported causes of sarcopenia are decreases in growth hormones and increases in cytokines. Meanwhile, the differentiation between sarcopenia and the atrophy of disused muscles is unclear and their clear differentiation will be important in future studies. Recently, the diagnostic criteria of sarcopenia have been defined according to a large-scale investigation. In the future, an easier method of diagnosing sarcopenia should be developed. Specific treatment strategies more closely correlated to the clinical condition of individual patients must be designed, because the causes of sarcopenia vary widely. We attempted to summarize the characteristics of the clinical condition, diagnosis, and treatment of sarcopenia.

Publications

*Kimura M*¹, *Iida M*¹, *Yamauchi H*, *Suzuki M*, *Shibasaki T*, *Saito Y*¹, *Saito H*¹ (*'Keio Univ)*. Astaxanthin supplementation effects on adipocyte size and lipid profile in OLETF rats with hyperphagia and visceral fat accumulation. *J Funct Foods*. 2014; **11**: 114-20.

Reviews and Books

Udaka J, Fukuda N, Yamauchi H, Marumo K. Clinical definition and diagnostic criteria for sarcopenia. Journal of Physical Fitness and Sports Medicine. 2014; **3:** 347-52.

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. Research on visual stimulus and posture control

Information for maintaining body direction and movement of the body center for maintaining posture are determined by visual input factors, equilibrium vestibular input factors, and somatosensory factors from the whole body (including muscles, tendons, joints, and skin). Visual information becomes the main factor in outer space because vestibular and somatosensory inputs are reduced owing to low or absent gravity. The objective of this research is to analyze changes in posture induced by visual stimuli.

2. The 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.

To accomplish the above objective, astronauts who have stayed 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

In the present experiment, no measurements were made in orbit; instead, measurements were made only before and after a stay in orbit. The aim is to use the resulting data as the basis for a better understanding of the physiological issues that occur in skeletal muscles and the somatosensory system because of a simulated space environment or long-term space flight and apply it to create a more effective training and rehabilitation course.

For astronauts who stay in space for a long time, the following are measured before and after their stay: (1) electromyography of the hind limb antagonistic muscles (the tibialis anterior and soleus, gastrocnemius, and plantaris muscles), (2) blood flow in the lower limb skeletal muscles by using a blood flow measuring device, and (3) the relationship between the nervous system and hind limb skeletal muscles in body-sway balance control by tilting their center of gravity on a force plate in a direction displayed by an image.

3. Biomedical analyses of human hair exposed to long-term space flight

The purpose of this research is to study the effects of long-term exposure in space flight on gene expression and mineral metabolism in human hair. In this experiment, we plan 2 analyses of human hair from International Space Station crews. These 2 analyses are (1) nucleic acids (RNA and mitochondrial DNA) and (2) minerals (Na, K, and Ca) and a trace element (Hg) in the hair shaft. The experiment requires in total 10 crew members of the International Space Station who will stay in orbit more than 90 days. During this experiment, hair samples will be collected a total of 6 times (2 times each in preflight, in flight, and in postflight periods) for each subject.

Hair matrix cells actively divide in a hair follicle and sensitively reflect physical conditions. The hair shaft has an advantage in that it records the metabolic conditions of the environment where the subject is. These samples give us useful physiological information to examine the effect of space flight. In space experiments, we believe that hair is one of the most suitable biological specimens because there are no special requirements for utilizing hardware and handling.

4. Outreach activities for aerospace medicine

Our outreach activities aim to promote public understanding of science and to provide information to the public by publishing books and holding public talks, lectures, and discussions.

Publications

Nagatomo F, Terada M, Ishioka N, Ishihara A. Effects of exposure to microgravity on neuromuscular systems: a review. International Journal of *Microgravity Science and Application.* 2014; **31:** 66-71.

Department of Pathology Division of Neuropathology

Masahiro Ikegami, Professor and Director Junko Fujigasaki, Assistant Professor 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

Accumulation of subunit c of mitochondria ATP synthase in the central and peripheral nervous systems in human lysosomal diseases

Objective: This study investigated the accumulation of subunit c of mitochondria ATP synthase (SCMAS) in the central nervous system in lysosomal disorders.

Material and methods: We used SCMAS immunohistochemistry to analyze the central and peripheral nervous systems of Niemann-Pick disease type C, mucopolysaccharidoses types I and II, neuronal ceroid lipofuscinoses, Gaucher disease, Fabry disease, mucolipidoses types II and III. An antibody against SCMAS was raised in rabbits with keyhole limpet hemocyanin-fused DIDTAAKFIGAGAATVGVAC. An affinity-purified anti-SCMAS antibody was purified from rabbit sera with glutathione S-transferase-DIDTA-AKFIGA binding column.

Results: In the central and peripheral nervous systems of lysosomal disorders, the numbers of SCMAS-immunoreactive neurons increased in proportion to the amico-cupuricsilver-impregnated neurons.

Discussion: SCMAS is reported to accumulate in the neuronal cytoplasm of neuronal ceroid lipofuscinoses and mucopolysaccharidoses types I and II. We found neuronal SCMAS accumulation in the central and peripheral nervous systems of Niemann-Pick disease type C, Fabry disease, and mucolipidoses. The accumulation of SCMAS suggests that a disturbance of ATP synthase might cause the neuronal deaths in lysosomal disorders.

A biopsy case with Nocardia farcinica brain abscess

A 72-year-old man with Gerstmann syndrome had left parietal ring-enhanced lesions in magnetic resonance images of the brain. Formalin-fixed paraffin-embedded (FFPE) tissue of the lesion showed branching filamentous rod-shaped bacteria marked with Grocotte, Gram, and Kinyoun stains. To identify bacteria, we applied 16S ribosomal RNA (rRNA) gene sequencing in FFPE tissue. The extraction of DNA was performed with the GeneRead DNA FFPE kit (Qigen, Venlo, the Netherlands) following the manufacturer's
instructions. Primers (5'-GTTTGATCCTGGCTCA-3' and 5'-TACCAGGGTATCTA-ATCC-3') were used for the first 800 bp and primers (5'-GGATTAGATACCCTGGTA-3' and 5'-CGGTTACCTTGTTACGACTT-3') were used for the last 800 bp of the 16S-rRNA gene. The annealing temperature was 58°C. BigDye Terminator v3.1 cycle sequencing kits and an ABI PRISM 310 genetic analyzer (Thermo Fischer Scientific, Waltham, MA, USA) were used to determine DNA sequences. The 16S rRNA gene sequences obtained were compared with the GenBank databases (http://www.ncbi.nlm. gov/BLAST/) and identified as being from the bacterial species Nocardia farcinica. The bacterial identification rates for paraffin tissue sections were 18% to 70%. For the treatment of bacterial infection, the 16S-rRNA DNA sequences of bacterial species should be evaluated in lesions.

A 132-day-old male neonate with ectodermal dysplasia examined at autopsy

A male neonate was born to a 40-year-old gravida 1 mother after frozen embryo transfer. The pregnancy was complicated by umbilical artery regurgitation and amniotic membrane peeling, and the child was delivered prematurely with cesarean section at 32 weeks' gestation. Birth weight was 1,089 g. Multiple congenital malformations were noted at birth and included alopecia of the scalp, anhydrosis, hyperkeratosis, inguinal herniation, and a ventricular septal defect. The neonate died of sepsis, and autopsy revealed enlarged lateral cerebral ventricles with periventricular white matter necrosis. The results of exome sequencing identified mutations of epidermal growth factor receptor as a cause of ecto-dermal dysplasia.

Publications

Sengoku R, Matsushima S, Murakami Y, Fukuda T, Tokumaru AM, Hashimoto M, Suzuki M, Ishiwata K, Ishii K, Mochio S. ¹¹C- PiB PET imaging of encephalopathy associated with cerebral amyloid angiopathy. *Intern Med.* 2014; **53:** 1997-2000.

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 professionals), 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 2014 we have been mostly focused on basic research.

Research Activities

A questionnaire for ankle sprain in elite ballet dancers

We investigated the incidence and risk factors of ankle sprain in elite ballet dancers with a questionnaire we developed. The study group consisted of 28 male and 105 female dancers. When these dancers were aged 11 to 15 years, 56% had an ankle sprain and 50% had bilateral ankle sprains. Dancers who had used pointe shoes before the age of 10 years tended to have more than one injury and complained of instability of the ankles. Ballet dancers appear to need measures to prevent ankle sprains based on specific risk factors.

Correlation of deep squatting and sitting ability with the incidence of sports injuries of the lower extremity in baseball and soccer players: A prospective study

We performed a prospective study to examine possible correlations of deep squatting and sitting position ability with the incidence of sports injuries in the lower extremity in baseball and soccer players. The subjects were 30 patients in each group with an average age of 18 years. The correlation (phi coefficient) of deep squatting with the incidence of sports injuries of the lower extremity was 0.94 in baseball players and 0.78 in soccer players. The correlation (phi coefficients) of deep sitting with the incidence of sports injuries was 0.48 in baseball players and 0.47 in soccer players. The ability to perform deep squatting can be easily examined and can serve as a useful evaluation method to predict future sports injuries.

Therapeutic effects of high molecular weight hyaluronan injections for tendinopathy in a rat model

We analyzed the therapeutic effects of hyaluronan injections for tendinopathy in a rat model, which was developed with a rodent treadmill machine. Rats with tendinopathy received injections of high molecular weight hyaluronan, normal saline, or nothing (control group) into the space between the patellar tendon and the fat pad of both knees. The number of spontaneous locomotor activities was significantly greater after the injection of hyaluronan than of normal saline or nothing. On histologic examinations, the numbers of microtears, laminations, or apoptotic cells in patellar tendons were significantly lower after the injection of hyaluronan than of normal saline or nothing. Injections of high molecular weight hyaluronan were effective for pain relief and for partial restoration of the patellar tendon in our tendinopathy rat model.

A junior high school baseball player with bilateral anterior iliac pain of independent pathology: A case study

We reported on a 14-year-old baseball player who sustained bilateral anterior iliac pain of independent pathology: overuse syndrome on the right side and iliacus hematoma on the left. We speculated that early diagnosis with magnetic resonance imaging was effective for preventing serious complications, such as an avulsion fracture and femoral nerve palsy.

Arthroscopic reduction and internal fixation for fracture of lateral process of the talus We reported on a 22-year-old snowboarder who had a type I fracture of the lateral process of the talus and underwent arthroscopic reduction and internal fixation. This was the first report describing the arthroscopic approach for treatment of this fracture.

Publications

Funasaki H, Kato S, Hayashi H, Marumo K. Arthroscopic excision of bone fragments in a neglected fracture of the lateral process of the talus in a junior soccer player. *Arthrosc Tech.* 2014; **3:** e331-4.

Hayashi H, Funasaki H, Sakamoto K, Tsuruga R, Marumo K. Recovery in muscle strength after anterior cruciate ligament reconstruction (in Japanese). Nihon Seikeigeka Supotsu Igakkai Zasshi. 2014; **34:** 322-8.

Ito S, Funasaki H, Hayashi H, Kawai K. Com-

parison of silent period between the operated and non-operated side after reconstruction of anterior cruciate ligament (in Japanese). *JOSKAS*. 2014; **39:** 821-5.

Reviews and Books

Sugiyama H, Kim S. Sports injuries in the pelvis and hip joints (in Japanese). *MEDICAL REHABILI-TATION*. 2014; **176**: 155-62.

Department of Allergology

Naohiro Watanabe, Professor and Director

Hirohisa Saito, Professor

General Summary

Our research concerns the biological significance of immunoglobulin E (IgE) and mechanisms of protection against parasites.

Research Activities

Protection against reinfection with Vampirolepis nana eggs

Vampirolepis nana, dwarf tapeworm, is a human parasite that also infects mice. Oral infection with eggs of V. nana induces strong protection against reinfection with eggs in the small intestine of mice. The protection is induced within 2 days after primary infection through innate immunity and 1 week after primary infection through acquired immunity. The mechanisms of protection were examined in innate and acquired immunity. Our previous study indicated that CD4T cells are responsible for protection in innate immunity. In addition, collaboration of CD4 bearing $\alpha\beta T$ cells and $\gamma\delta T$ cells are essential, and costimulatory signals from inducible T-cell costimulatory ligand (ICOSL) are required. Cytokines, such as interleukin (IL)-4 and IL-13, but not interferon γ and IL-12, are involved in innate immunity. Protection through innate immunity is induced by complicated interaction between cells and molecules within a very short period of time. Although innate immunity generally acts against primary infection, the protection against V. nana eggs acts against secondary infection, but not to primary infection. These findings suggest a novel innate immune system in the small intestine. On the other hand, an experimental system was established to evaluate protection against reinfection with eggs through acquired immunity. Mice were reinfected with eggs 4 weeks after primary infection. The protection through acquired immunity is induced by CD4-bearing $\alpha\beta T$ cells. Acquired immunity is known to depend on CD4-bearing memory T cells. Moreover, signals from ICOSL, IL-4, and IL-13 are not required. Different mechanisms are considered between acquired and innate immunity in the phase of protection against larvae from eggs.

Publications

Velasquez CV¹, Roman AD¹, Lan NTP², Huy NT¹, Mercado ES³, Espino FE³, Perez ML⁴, Huong VT², Thuy TT⁵, Tham VD⁶, Nga CT⁶, Ha TT², Bilar JM⁴, Bajaro JD⁴, Baello BQ⁴, Kikuchi M¹, Yasunami M¹, Morita K¹, Watanabe N, Karbwang J¹, Hirayama K¹ (¹Nagasaki Unv, ²Pasteur Inst, ³Res Inst Trop Med, ⁴Philippine Child Med Ctr, ⁵Hosp No. 2, ⁶Ctr Prev Med). Alpha tryptase allele of *tryptase* 1 (*TPSAB1*) gene associated with dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) in Vietnam and Philippines. *Hum Immunol.* 2015; **76:** 318-23. Epub 2015 Mar 20.

Reviews and Books

Watanabe N. Parasitic diseases (in Japanese). In: Arakawa Y, Kamiya S, Yanagi U, editors. Medical microbiology. Tokyo: Tokyo Kagaku Dojin; 2014. p. 215-20.

Department of Pathophysiology and Therapy in Chronic Kidney Disease

Tatsuo Hosoya, Professor Iwao Ohno, Professor Keitaro Yokoyama, Associate Professor Satoru Kuriyama, Professor Kimiyoshi Ichida, Professor Yukio Maruyama, Assistant 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 IIIab and hyperuricemia.

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 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 status of PD coordinators. 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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Research Center for Medical Sciences

The Research Center of Medical Sciences was reorganized in 2014 to promote research in our university. Now, the Center has been divided into 3 groups, which are the Research Support Unit, the Research Unit, and the Research Institute, and most functions of the Center were gathered in University Building No. 1. The Research Support Unit consists of 5 facilities, which are the Core Research Facilities for Basic Science (Molecular Genetics), the Core Research Facilities for Basic Science (Molecular Cell Biology), the Laboratory Animal Facilities, the Radioisotope Research Facilities, and the GMP Production Facilities for Cell Therapy and Gene Therapy. The Research Units consist of 9 divisions, which are the Division of Gene Therapy, the Division of Oncology, the Division of Molecular Immunology, the Division of Medical Engineering, the Division of Neuroscience, the Division of Clinical Pharmacology and Therapeutics, the Division of Molecular Epidemiology, the Division of Clinical Epidemiology, and the Division of Regenerative Medicine. Each division is performing original research. Two research institutes exist in the Center: the Institute for High Dimensional Medical Imaging in the Kokuryo campus and the Institute of Clinical Medicine and Research in the Kashiwa campus. The details of educational and research activities in each facility, division, and institute are described below. Now the system of research support in our university and progress of own research has been established.

Division of Gene Therapy

Toya Ohashi, Professor and Director

Hiroshi Kobayashi, Associate Professor

General Summary

As we did last year, this year we have been studying lysosomal storage diseases (LSDs) and various cancers of the digestive tract. In the research on LSDs, we have been developing novel gene therapy technology, novel strategies to overcome limitations of current therapies (enzyme replacement therapy [ERT] and bone marrow transplantation [BMT]), pathophysiological analysis of LSDs using induced pluripotent stem (iPS) cells and molecular analysis of patients with LSDs. In the research on cancers of the digestive tract, we have been developing a novel gene therapy method using a protease inhibitor.

Research Activities

Immune tolerance induction of ERT for LSDs

Animal and human studies of ERT for Pompe disease have indicated that antibodies generated against infused recombinant human α -glucosidase (GAA) can have a negative effect on the therapeutic outcome and cause hypersensitivity reactions. We have previously shown that oral administration of GAA reduces immune tolerance against GAA. In a previous study, wild-type mice were immunized after receiving only 2 intraperitoneal injections of GAA/adjuvant. To study whether the above approach for inducing immune tolerance can be applied to clinical ERT, we mimicked the clinical protocol. As a result, the oral administration of GAA efficiently prevented lethal hypersensitivity and successfully induced immune tolerance.

BMT for LSDs

Mucopolysaccharidosis (MPS) type II is a lysosomal storage disorder caused by deficient activity of the iduronate-2-sulfatase (IDS). Although BMT has been proposed to have a beneficial effect for patients with MPS II, the requirement for donor-cell chimerism to reduce glycosaminoglycan (GAG) levels is unknown. To address this issue, we transplanted various ratios of normal and MPS II bone marrow cells in a mouse model of MPS II and analyzed GAG accumulation in various tissues. The level of GAG reduction in these tissues depends on the percentage of normal-cell chimerism. These observations suggest that a high degree of chimerism is necessary to achieve the maximum effect of BMT. However, the relation between clinical responses and the level of GAG reduction should be clarified.

Gene therapy using lentiviral vector system and homologous recombination using zinc finger methods for LSDs and the analysis of bone system metabolism in MPS II We are investigating the effect of gene therapy for MPS VII, MPS II, and Krabbe disease. For MPS VII, we injected a lentiviral vector into newborn mice and found that this treat-

ment increased β -glucuronidase expression and decreased accumulated GAGs in key organs at 20 to 30 weeks, increased the vector copy number in the brain at 30 weeks, and decreased the autophagic buildup in the brain. We performed *ex vivo* gene therapy in a mouse model of MPS II at 8 weeks of age using a recombinant lentiviral vector expressing the IDS gene (*IDS*). This treatment succeeded in establishing long-term overexpression of the IDS enzyme in the circulating blood and efficient IDS expression and decreasing levels of accumulated GAGs in the brain. (Wakabayashi et al. 2015 Human Gene Therapy)

We have begun to analyze bone system metabolism, including factors of signal transduction, in MPS II and will investigate the effect of gene therapy using lentivirus vector.

For Krabbe disease, we performed neonatal gene therapy using a lentiviral vector and succeeded in increasing the expression of β -galactosylceramidase (GALC), decreasing high levels of accumulated psychosine in the brain, improving myelin-forming cells, and increasing life spans. We also attempted to use the zinc finger system for site-specific homologous recombination in the GALC gene (*GALC*) *in vitro* and succeeded in exchanging a specific sequence of *GALC* and increasing GALC expression in the treated cells. These results suggest the efficient effect of the neonatal gene therapy and the zinc finger system for LSDs.

Development of a method for measuring disease-specific iduronic acid from the nonreducing end of GAGs in MPS II

The GAGs are used as a biomarker for analyzing MPSs, including MPS II. However, the conventional assay for total GAGs has low sensitivity and specificity for evaluating abnormalities in tissue extracts of MPS II. To resolve this problem, we developed a novel method of measuring disease-specific GAGs based on the analysis of 2-sulfoiduronic acid levels derived from the nonreducing end of GAGs by using recombinant human IDS and iduronidase. Our method demonstrated that the levels of generated iduronic acid were markedly increased in tissues from MPS II mice, whereas the monosaccharide was not detected in samples from wild-type mice. This result indicates that this assay is useful for analyzing disease-specific GAGs in tissues from MPS II mice.

Identification of cryptic novel α -galactosidase a gene mutations: Abnormal messenger RNA splicing and large deletions

Fabry disease is an X-linked lysosomal storage disorder caused by mutation of the galactosidase alpha (GLA) gene (GLA) and resulting in the deficient activity of GLA. This deficient activity causes various glycolipids, such as globotriaosylceramide, to be stored in many tissues. The main clinical symptoms of Fabry disease are neuropathic pain, hypohydrosis, and cerebrovascular, renal, and cardiac disease. Although Fabry disease is inherited in an X-linked recessive manner, clinical symptoms often develop in heterozygous female patients. In male patients, enzyme analysis is usually sufficient for diagnosis. However in female patients, especially those without a family history of the disease, a definitive diagnosis often requires the pathologic GLA mutation to be detected. More than 700 variants and mutations have been identified in GLA from patients with Fabry disease. In the present study, we examined GLA from patients with Fabry disease who did not carry mutations in exons or exon/intron boundaries. We found some novel cryptic mutations related to Fabry disease: a multiple-exon deletion mutation, an insertion mutation of splicing enhancer sequence, an insertion mutation of exon-skipping element by long interspersed nuclear element 1 retrotransposon element, and a point mutation in a nonprotein coding region by MLPA (multiplex ligation-dependent probe amplification) method and analyzing cDNA.

Gene transfer and metabolic profiling of Pompe disease induced pluripotent stem cells

We have investigated late-onset Pompe disease-specific induced pluripotent stem cells and have already shown that cardiac differentiation is useful in terms of the disease modeling. We have generated a third-generation lentiviral vector expressing missing enzyme and confirmed the dose-dependent expression in patient-specific induced pluripotent stem cells. Moreover, the enzyme activity and glycogen accumulation also improve according to the lentiviral gene transfer. Therapeutic efficacy is maintained even after the cardiac differentiation. Next, we have quantified the metabolic status of Pompe disease-specific induced pluripotent stem cell-derived cardiomyocyte by "metabolomic analysis." Pompe disease-induced pluripotent stem cell-derived cardiomyocytes show the oxidative stress. We have also validated that such change occurs in the cardiomyocytes and skeletal muscle in the mouse model of Pompe disease. Oxidative stress might be associated with the mechanism of Pompe disease *in vitro* and *in vivo*. In the future we plan to assess the efficacy of antioxidant in the pluripotent stem cell-derived cardiomyocytes and model mice.

Antitumor effect of nafamostat mesilate for digestive cancer and treatment of cancer pain Recent studies have demonstrated that nuclear factor (NF)- κ B plays important roles in the regulation of cell apoptosis, inflammation, and oncogenesis. Inhibition of NF- κ B is a potential new strategy for the treatment of cancers. We have previously reported that nafamostat mesilate, a serine-protease inhibitor, inhibits NF- κ B activation 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 or gemcitabine/nanoparticle albumin-bound paclitaxel, we investigated the combination therapy of these anticancer agents and NF- κ B inhibitor. Moreover, we have investigated the antitumor effect of combination therapy with nafamostat mesilate and radiation for pancreatic cancer.

Ionizing radiation enhances epithelial-mesenchymal transition (EMT) and cancer metastasis. Neoadjuvant chemoradiation for colorectal cancer also enhanced EMT. Therefore, we have suppressed EMT by inhibiting NF- κ B 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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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 researchers, would be verified by basic and clinical studies so that they could be applied clinically. Most of our research has been based on antitumor immunity.

Research Activities

Nafamostat mesylate inhibits interferon-gamma-induced expression of programmed cell death ligand 1 on cancer cells

As the interaction between programmed cell death (PD) 1 on cytotoxic T lymphocytes (CTLs) and PD ligand (PD-L) 1 on cancer cells induces the apoptosis of CTLs, PD-1/PD-L1 interaction plays an important role on the escape of cancer cells from antitumor immunity. The expression of PD-L1 on cancer cells is induced by interferon gamma produced by CTLs, which attack cancer cells. We found that treatment of cancer cells with nafamostat mesylate, a serine protease inhibitor, significantly suppressed interferon-gamma-induced PD-L1 expression on cancer cells in vitro at both messenger RNA and protein levels. Combined treatment of the cancer patients with anti-PD-1 antibodies and nafamostat mesylate might become promising for cancer.

Immunotherapy against glioblastoma with a dendritic cell vaccine

The clinical effect of the combination therapy with temozolomide and a dendritic cell (DC) vaccine against glioblastoma was examined. Patients with glioblastoma were assigned to 2 groups: a group with recurrent glioblastoma after failing temozolomide chemotherapy and a group with newly diagnosed glioblastoma. Autologous cultured glioma cells obtained from surgical specimens were fused with DCs using polyethylene glycol treatment. Progression-free survival and overall survival in patients with recurrent glioblastoma (n = 9) or newly diagnosed glioblastoma (n = 20) treated with the DC vaccine were significantly longer than those untreated with the DC vaccine. Up-regulation and cytoplasmic accumulation of chemoresistance-associated peptides were observed in recurrent glioblastomas. Specific immune responses against chemoresistance-associated peptides.

Generation of artificial antigenic protein inducing potent cellular immunity

Invocation of cellular immunity by epitopic peptides remains largely dependent on empirically developed protocols, such as interfusion of aluminum salts and emulsification using terpenoids and surfactants. To explore novel vaccine formulation, epitopic peptide motifs were co-programed with structural motifs to produce artificial antigens with our "motifprogramming" approach. As a proof of concept, we used an ovalbumin system and prepared an artificial protein library with combinatorially polymerizing major histocompatibility complex (MHC) class I and II sequences from ovalbumin along with a sequence that tends to form secondary structures. The purified endotoxin-free proteins were then examined for their ability to activate ovalbumin-specific T-cell hybridoma cells after being processed within DCs. One clone, F37A (containing 3 MHC I and 2 MHC II ovalbumin epitopes), possessed a greater ability to evoke cellular immunity than did the native ovalbumin or the other artificial antigens. The sensitivity profiles of drugs that interfered with the F37A uptake differed from those of the other artificial proteins and ovalbumin, suggesting that alteration of the cross-presentation pathway is responsible for the enhanced immunogenicity. Moreover, F37A, but not an epitopic peptide, invoked cellular immunity when injected together with monophosphoryl lipid A and retarded tumor growth in mice. Thus, an artificially synthesized protein antigen induced cellular immunity in vivo in the absence of incomplete Freund's adjuvant or aluminum salts. The method described here might be used to develop vaccines for such intractable ailments as acquired immunodeficiency syndrome, malaria, and cancer, ailments in which cellular immunity likely plays crucial roles in prevention and treatment.

Whole exome-based search for cancer-specific antigens recognized by T-cell immunity

Cancer cells have many genetic alterations that generate amino acids changes of proteins. Antigenic peptides on the human leukocyte antigen (HLA) from these altered proteins are thought to be cancer-specific epitopes. Whole exome analysis of the cancer cell was performed, and the HLA-binding capacity of altered peptides was analyzed to investigate whether the altered peptide has high HLA-binding capacity. Variant peptides whose binding capacity to HLA was predicted to be stronger than that of original peptides were found in prostate cancer cell lines. These variant peptides might be more cancer-specific than the original peptide and possibly become targets of antitumor immunity. A whole exome-based search method for variant peptides with high HLA-binding capacity might provide the immune-responsive cancer-specific antigens of individual patients with cancer.

Intensified rituximab therapy against diffuse large B-cell lymphomas mediated by CD20 up-regulation by gemcitabine treatment

Treatment with gemcitabine increased CD20 expression by cells from human diffuse large B-cell lymphomas (DLBCLs) in vitro at messenger RNA and protein levels. This CD20 up-regulation is closely associated with activation of nuclear factor kB induced by gemcitabine. The rituximab-mediated complement-dependent cytotoxic activity against gemcitabine-pretreated DLBCL cells was enhanced by the increased binding of rituximab. Combined treatment with gemcitabine and rituximab might become a promising therapy against DLBCLs.

Soluble PD-L1 as an important mediator for immunosuppression in patients with pancreatic cancer

We found abundant soluble PD-L1 (sPD-L1) in the plasma of patients with advanced pancreatic cancer. The concentration of plasma sPD-L1 was decreased along with the decline of the lymphocyte number induced by chemotherapy. Furthermore, PD-L1 was highly expressed on CD4+T cells in patients with pancreatic cancer, suggesting that sPD-L1 was derived from CD4+T cells in blood. The CD4+T cells from the patients with pancreatic cancer release sPD-L1 into the medium in vitro. If the CD4+T cells in these patients could generate sPD-L1 that is functional enough for CTL suppression, antitumor immunity should be inhibited in the tumor microenvironment in which tumor PD-L1 inactivates PD-1 expressing CTLs and also in the peripheral blood in which sPD-L1 binds to PD-1 expressing CTLs.

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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

Repeated administration of interleukin 31 upregulates interleukin 31 receptor A in dorsal root ganglia and causes severe itch-associated scratching behavior in mice We investigated the effects of repeated administration of interleukin (IL) 31 on itch-associated scratching counts (long-lasting scratching) and IL-31-related receptor messenger RNA expression in mice. Intradermal injection of IL-31 (100 and 300 ng/site) every 12 hours for 3 days significantly increased long-lasting scratching. Repeated administration of IL-31 also increased the expression of IL-31 receptor A (IL-31RA) and oncostatin M receptor beta in dorsal root ganglia (DRG). After the repeated administration of IL-31 was discontinued, IL-31RA expression decreased and reached the baseline level 2 days after the last dose of IL-31. Long-lasting scratching changed along with DRG IL-31RA expression. Moreover, IL-31-induced IL-31RA protein expression was confirmed with Western blotting analysis. These data suggest that IL-31 upregulates IL-31RA expression in DRG neuron cell bodies and that cutaneously injected IL-31-induced itching is enhanced by DRG IL-31RA expression in mice.

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. However, for inducing oral tolerance the administration of high-dose antigens is required. Recent technical progress has allowed the production of transgenic rice that accumulates antigens at high concentrations. The aim of this study was to investigate whether oral immunotherapy with a transgenic rice seed containing a hybrid peptide (7Crp) comprising 7 human major T-cell determinants in Japanese cedar pollen allergens, Cry j 1 and Cry j 2, is safe and effective to induce oral tolerance in patients with Japanese cedar pollinosis. A blinded, randomized, placebo-controlled trial employing oral immunotherapy with 80 g of steamed pack rice for cedar pollinosis was performed for 20 weeks. Thirty subjects were enrolled and divided into 2 groups that ate transgenic rice or normal rice. No major adverse effects were observed in either group during treatment. Allergen-specific T- and B-cell responses were evaluated. The ratio of allergen-specific T cells proliferative responses to 7Crp peptide, Cry j 1, and Cry j 2, which gradually increase in pollen season, were significantly lower in subjects who ate transgenic rice than in subjects who ate normal rice. On the other hand, the ratio of allergen-specific T cells proliferative responses to purified protein derivative antigen, which is used for the tuberculin skin test, did not differ between the groups. Furthermore, allergen-driven IL-5 and IL-13 were also significantly reduced in culture supernatants of peripheral blood mononuclear cells after subjects had eaten transgenic rice.

Adjuvant for inducing antigen-specific cytotoxic T lymphocytes via cross-presentation of cationic lipids

To elicit immune responses against tumors and viruses, vaccines that can induce antigenspecific cytotoxic T lymphocytes (CTLs) are most promising. However, administering protein antigens with commonly used adjuvants fails to induce antigen-specific CTLs. In this study, to obtain an adjuvant that could induce CTLs when protein antigens were used, we prepared cationic liposomes with dimethyldistearylammonium bromide. We assessed this adjuvant's efficacy by focusing on its activity to induce epitope-specific CTLs in mice with chicken ovalbumin protein in vivo.

This optimized adjuvant rapidly induced epitope-specific CTLs directed against protein antigens. The stability of dimethyldistearylammonium-containing liposomes was significantly improved by the addition of several reagents. In particular, the addition of some detergents improved the stability of liposomes after protein antigens and adjuvants were mixed. Furthermore, this adjuvant could efficiently induce CTLs simply by immediately being mixed with an antigen and without encapsulation. Without encapsulation, larger amounts of protein antigens were immunized than with encapsulation. The induction of CTLs was independent of Toll-like receptor ligand 2, 4, and 9 signaling and helper T cells. This adjuvant provided for strong CTL induction within 5 days in mice, and CTL also exhibited inhibitory effects against ovalbumin protein epitope-expressing tumors. With a single vaccine using an extract from B16 melanoma cells in conjunction with this adjuvant, melanoma growth and metastasis were significantly suppressed. Thus, this adjuvant should be useful for studies of CTL induction and for preparing T-cell vaccines against tumors with unknown MHC class I epitopes.

Publications

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Division of Medical Engineering

Masayuki Yokoyama, Associate Professor and Director

Kouichi Shiraishi, Assistant Professor

General Summary

The Medical Engineering Laboratory provides new and essential techniques for developments of medical treatment. There are 2 key technologies in our laboratory: ultrasound and polymer nanomedicine. We have developed sonothrombolysis for treating acute ischemic stroke. For this project for acute ischemic stroke, we have collaborated closely with clinical departments and basic science laboratories, both in our university and hospitals and others. For the other key technology, polymer nanomedicine, we have applied polymer micelles carrying various drugs, such as anticancer drugs and diagnostic drugs, for cancer treatment and acute ischemic treatment. We have applied a polymeric micelle system to a magnetic resonance imaging (MRI) contrast agent for the diagnosis of cancers and acute ischemic stroke. The polymeric micelle carrier system has a great potential for therapy when it is combined with the diagnosis of cancers and acute ischemic stroke.

Research Activities

Medical application of ultrasound

We have applied transcranial ultrasound for the thrombolysis of acute ischemic stroke (sonothrombolysis). For this condition, tissue plasminogen activator (t-PA) treatment within 4.5 hours of the stroke onset is the only effective thrombolytic therapy. Therefore, a safe and effective technology to enhance the therapeutic effects of t-PA would be highly beneficial. We have shown that transcranial ultrasound for thrombolysis can enhance the thrombolytic activity of t-PA and increase the recanalization rate. Although the recanalization rate is increased, other groups have shown that standing waves at an ultrasound frequency of 300 kHz induced a high risk of brain hemorrhage. Therefore, we have applied ultrasound at a medium frequency (500 kHz), which possess a greater thrombolytic effect than the standard diagnostic frequency (2 MHz) and is safer than a frequency of 300 kHz. However, we should evaluate the hemorrhage risk of transcranial ultrasound at 500 kHz. We have found that our modulation method, which involves periodic selection of random ultrasound frequencies in the range of 400 to 600 kHz, reduces standing waves. We have developed an instrument that can determine the effect of sonothrombolysis through the absorption of blood clots. With this instrument, we have obtained sound intensity-dependent clot lysis with t-PA treatment.

Polymeric micelle drug carrier systems

Self-assembly nanoparticulates have been actively examined for drug targeting. Professor Yokoyama, the director of this laboratory, is an international pioneer in the development of self-assemblies of synthetic block copolymers and polymeric micelles for anticancer drug targeting systems. Currently, 4 formulations of polymeric micelle anticancer drugs are undergoing clinical trials in Japan, Europe, and the United States. We are working to develop the next generation of novel technology based on the polymeric micelle carrier systems.

We have developed a new polymeric micelle MRI contrast agent for the diagnosis of solid tumor. We have shown that this MRI contrast agent possesses the ability to target solid tumor tissues and exhibits high signal intensity in small solid tumors. We have been studying a novel application of the MRI contrast agent for acute ischemic stroke. In a 3-hour middle cerebral artery occlusion (MCAO) model, the MRI contrast agent quickly showed high signal intensity within part of ischemic core in the hemisphere. The high signal intensity area did not completely overlap with the high signal intensity ischemic core where both diffusion-weighted images and T_2 -weighted images provided high contrast. Furthermore, the images obtained with the MRI contrast agent were not obtained with a conventional low-molecular-weight MRI contrast agent. Because the image provided a damaged area of the blood-brain barrier where hemorrhage might occur after treatment with t-PA, the polymeric micelle MRI contrast agent system has a great potential to assess the hemorrhage risk of ischemic stroke. The MRI contrast agent must be further optimized to be suitable for this purpose. We proved that polymeric micelle carrier system delivers into the ischemic hemisphere beyond the blood-brain barrier; therefore, the polymeric micelle carrier system will be useful in both the diagnosis and treatment of acute ischemic stroke. This is our new and valuable challenge.

We have been studying synthetic polymer-related immune responses. The phenomenon exhibits specificity for poly(ethylene glycol) (PEG), which is used for a polymeric micelle drug carrier. The PEG-specific antibody (anti-PEG IgM) is generated when either PEG-PBLA micelles or PEGylated liposomes are intravenously injected. However, we have found that the behaviors of polymeric micelles are very different from those of PEGylated liposomes, although both nanoparticulates possess PEG. The polymeric micelles exhibited little or no change in pharmacokinetics in the presence of anti-PEG immunoglobulin M, whereas PEG liposomes exhibited drastic change in pharmacokinetics. We further evaluated the effect of the anti-PEG antibody on the behaviors of both polymeric micelles and PEGylated liposomes. We found that a limited number of anti-PEG IgM molecules had been generated after the priming. In contrast, polymeric micelle particles were 10 times as numerous as PEGylated-liposome particles, although the injected dose includes nearly the same number of PEG chains. We found 10 anti-PEG antibodies can bind to each PEGylated liposome, and antibody-PEGylated liposome complexes rapidly accumulate in the liver and spleen. Therefore, the polymeric micelle carrier systems have significant advantages for drug targeting in terms of the generated immune response.

We have tried to measure the inner core characteristics of polymeric micelles by means of synchrotron radiation (at the Super Photon Ring 8 Gigaelectronvolt facility, Hyogo Prefecture, Japan). The precise measurement accurately determined the inner core size of polymeric micelles, and we have shown a correlation between the structural characteristics of polymeric micelles and their in vivo and in vitro behaviors, in particular, their pharmacokinetics.

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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 channelrhodospin-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 transient pharmacological inactivation of the lateral parabrachial nucleus at the time of association significantly perturbs fear learning. We have also demonstrated that optogenetic 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.

Division of Clinical Pharmacology and Therapeutics

Shigeru Kageyama, Professor and Director

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: Survival data analysis (April 2014), Conflict of interest in medical research (July 2014), How to prevent misconduct in clinical trials (August 2014), and Ethical guidelines for medical and health care research involving human subjects (January 2015).

An administrative office for registration trials was established in the hospital in February 1999, and the system for registration trials in the hospital has been reformed to meet the

demands of the new good clinical practice guidelines. Seven clinical research coordinators (CRCs) now facilitate clinical trials. 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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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 2014, 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. Genome and epigenome clinical studies and lead findings
- 3. Elective class of global health
- 4. Randomized trial to prevent food allergy

Publications

Mafune A, Hama T, Suda T, Suzuki Y, Ikegami M, Sakanashi C, Imai S, Nakashima A, Yokoo T, Wada K, Kojima H, Urashima M. Homozygous deletions of UGT2B17 modifies effects of smoking on TP53-mutations and relapse of head and neck carcinoma. BMC Cancer. 2015; 15: 205. Takahashi GI, Otori Y, Urashima M, Kuwayama Y; for Quality of Life Improvement Committee. Evaluation of quality of life in Japanese glaucoma patients and its relationship with visual function. J Glaucoma. 2015 Mar 30. Epub ahead of print.

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Division of Clinical Epidemiology

Masato Matsushima, Professor and Director

General Summary

Division of Clinical Epidemiology is promoting the activity of clinical research, clinical epidemiology and education concerning them. Our specific aim is to support clinicians to solve their own problems in daily practice by epidemiological/clinical research skills.

The research themes of our division are medical communication, quality assessment of medical care, behavioral medicine, outcome research, qualitative research as well as disease-oriented epidemiological research. In particular, we aim to produce evidence in the field of primary-care due to the lack of evidence although primary-care is a front-line of practice.

As a contribution to the undergraduate education, our division holds classes of "Evidence-based clinical practice (EBCP)" to make medical students a skillful doctor being able to employ evidence-based approach.

Our postgraduate education concentrates on the methodology of clinical/epidemiological research and biostatistics. "The educational program for primary-care on clinical research methodology", which was started in 2007 by financial support of the Ministry of Education, Culture, Sports, Science and Technology in Japan was renewed as "Jikei Clinical Research Program for Primary-care" in 2009. Furthermore, as a subprogram of the project, "New Paradigms — Establishing Centers for Fostering Medical Researchers of the Future" supported financially by the Ministry of Education, Culture, Sports, Science and Technology in Japan, "Community Health and Primary Care Medicine" in the doctoral course was launched in 2014. The main aim of these programs is to make a primary-care physician a clinician-researcher.

Research Activities

EMPOWER-JAPAN study: Elderly Mortality Patients Observed Within the Existing Residence

Although little is known concerning the prognosis of patients receiving home medical care in Japan, there have been few prospective cohort studies of the elderly persons receiving the home medical care in Japan. EMPOWER-JAPAN study was started as a multi-centered 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 4-years. This study is financially supported by Japan Society for the Promotion of Science.

Comparison of diabetes care between specialists and general practitioners by the chronic care model

The chronic care model was developed during 1990's in the United States to improve the care of chronic illness by refining care-provider system, especially in a primary-care setting. The aim of this research is to clarify the usefulness of the chronic care model in Japan. The research plan consists of two steps. The first is to make the official Japanese version of the assessment form "Assessment of Chronic Illness Care" by following the World Health Organization procedure, for instance, translation, back translation and so on. This procedure has been finished. The second step is to compare the quality of diabetes care between specialists in diabetes and primary-care physicians as non-specialists.

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

The psychological impact on patients by disclosing lifestyle-related disease remains unknown. To clarify it, we compared the state of anxiety before and after the explanation of general checkup results by a cohort study in two primary-care facilities.

Cohort study of patient's complexity

As the size of the aged population increases, the patients' complexity on biomedical and psychosocial issues is thought to also increase. To examine the effect of patients' complexity on hospital admission such as length of stay, we started a cohort study at a regional hospital in Tokyo by employing the "Patient Centered Assessment Method".

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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. Disease models in genetically engineered mice are extremely useful but do not always precisely recapitulate the pathophysiology of human disease, especially neurodegenerative disorders. Good animal models will play a key role in studies leading to a greater understanding of the pathophysiology of neurodegenerative diseases. Recently, we have established a genetically modified primate model of human neurodegenerative disease. On the other hand, induced pluripotent stem (iPS) cell 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 modeling with iPS cells

We have generated 3 lines of iPS cells derived from patients with familial Parkinson's disease and optimized differentiation protocols for producing dopaminergic neurons from the iPS cells. Furthermore, we have started to generate iPS cells from patients with chronic renal disease and metachromatic leukodystrophy.

Function of neuronal Elav-like (Hu) proteins in embryonic and adult brain

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 (Hayashi et al. *J Neurosci Res.* 2015). 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.

Histological analyses showed accumulation of mitochondria and amyloid precursor protein in the swollen Purkinje axons, indicating that the axonal transport system might be impaired in HuC KO mice. To visualize mitochondrial dynamics in the axon, we infected Purkinje cells with a lentivirus encoding the photoconvertible fluorescent protein Kikume Green-Red, which targets the mitochondrion. Time-lapse imaging of mitochondrial migration revealed a disturbance of axonal transport in HuC KO mice. Furthermore, to identify HuC targets, we performed an RNA-binding protein immunoprecipitation-microarray (RIP-CHIP) assay and high-throughput sequencing-crosslinking immunoprecipitation (HITS-CLIP) assay. Isolated candidate RNAs include Kinesin family members KIF2A, KIF3A, and KIF3C, which are involved in axonal transport. Overexpression of KIF3A or KIF3C in Purkinje cells derived from HuC KO mice partially reversed the swelling of axons. These results indicate that, at least in part, the pathophysiological mechanism of axonal degeneration in HuC KO mice is due to the down-regulation of the kinesin proteins.

Electron microscopic analysis revealed that most of the spheroids of HuC KO mice were filled with mitochondria, smooth endoplasmic reticula, autophagosomes, and highly dense bodies. Amazingly, some of the spheroids were filled with nuclei and ribosomes, which should be in the cytoplasm. Mitochondria, smooth endoplasmic reticula, and autophagosomes are transported normally along axons and are essential for the homeostasis of neurons. However, the nuclei and ribosomes are not transported into axons. These results indicate that the pathological mechanisms of spheroid formation are not simply explained but are rather complicated.

Multimodal and exclusive pathology between amyotrophic lateral sclerosis and frontotemporal lobar degeneration caused by mutations of TDP-43

The 43-kDa transactive response DNA-binding protein (TDP-43) gene (*TDP-43*) has been identified as a causative gene of both amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Ubiquitin-positive cytoplasmic inclusion bodies containing TDP-43 are observed in neurons of patients with ALS or FTLD, and point mutations of the *TDP-43* gene have recently been found in both familial and sporadic cases of ALS and FTLD. However, the relationship between the pathogenesis of these conditions and the accumulation of inclusions is not clear, and even the multimodal/ exclusive linkage of ALS with FTLD has not been revealed.

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. We chose 2 mutants of the *TDP-43* gene, which were found in patients with either familial or sporadic ALS, by observing cultured cells holding multimodal structures of inclusions caused by overexpression of each mutant. Interestingly, considerable differences were observed in the phenotype and pathology between the 2 lines of mTDP-43 KI. Poor weight gain, decrease of motor function, and loss of motor neurons — 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. In the other line, decreases in anxiety levels, which may be related to FTLD, were observed, but not in another mTDP-43 KI mouse. These observations indicate that the mutation site is the predominant factor that causes multimodal or exclusive pathologies. Our results based on reference of *TDP-43* mutations are useful information for developing therapeutic strategies for ALS and FTLD.

A transgenic nonhuman primate model of neurodegenerative diseases

Medical research based on animal models serves as a bridge between basic and clinical research. Among various experimental animals, a nonhuman primate model is of growing importance for research in neuroscience and related fields, including pharmacology, genetics, reproductive biology, and social behavior. 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. We used a lentiviral vector to generate a transgenic marmoset carrying a mutant form of human alpha-synuclein (SNCA) and TDP-43. The SNCA gene is responsible for PARK1and PARK3-type Parkinson's disease with an autosomal dominant pattern of inheritance. On the other hand, TDP-43 is thought to be a causative gene of ALS. Lentivirus-transduced embryos were transferred to surrogate mothers, and founder animals were obtained. The founders were analyzed with minimally invasive methods, such as positron emission tomography and magnetic resonance imaging. In collaboration with Keio University, we developed a motion capture system capable of reconstructing 3-dimensional hand positions of common marmosets and evaluated hand kinematics during food retrieval movement in both healthy and hemispinalized animals (Takemi et al. Behav Brain Res. 2014). Advances in disease modeling using genetically modified primates will have a huge impact on future opportunities and progress in the research on neurodegenerative diseases.

A 9.4-tesla magnetic resonance imaging research system for magnetic resonance histology

Magnetic resonance histology is a powerful technology finely visualizing internal body structure and organs by dissecting at any plane in 3-dimensional imaging. Magnetic resonance imaging (MRI) has greatly extended the exploration of neuroanatomy, especially the wiring of nerve fibers in the brains of humans and animals. The quality of its images is guaranteed by high magnetic field MRI. Diffusion-tensor imaging is used to evaluate the anisotropic nature of water diffusion in tissues, and tractography might be used to evaluate 3-dimensional fiber structures.

Using a 9.4-T MRI, we visualized fine structures of human temporal bone, including Reissner's membrane; scala tympani, media, and vestibule; and nerve fibers. With diffusion-tensor imaging analyzed with an algorithm for tracking fibers, we successfully distinguished 4 separate nerves and tracked individual fibers to the end organ (cochlea, vestibules, and main trunk of facial nerve at the mastoid tip). This study was conducted in collaboration with Keio University and the Central Institute for Experimental Animals.

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

Hisashi Yamada, Professor and Director

General Summary

We can now analyze a person's whole genome within a week. Moreover, we can sequence the whole genome from a single cell. If we could perform sequencing without considering its ethical aspects, we could predict diseases that might affect the entire life of a new born baby. 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.

Research Activities

Cancer Molecular Biology

Cancers develop when mutations in specific genes accumulate in normal cells. Recent cancer research has disclosed that the disruption of epigenetic regulation in normal cells can also elicit cancers. Studying the changes of mutations in a patient's cancer during therapy, we can see how cancer cells acquire more malignant characteristics. We are studying the effects of anticancer agents on patients' cancer cells to detect mutations with the next generation of the high-throughput sequencer. We have applied this technique to a pediatric patient with double cancers, and were able to estimate the affected stem cells of both cancers. These findings will help reveal the etiology of cancer and lead to new treatments. This method was also applied for detecting genetic abnormality of mixed lineage leukemia-related leukemias, which are a group of leukemias with poor prognoses. By comparing the characteristics of several mixed lineage leukemia-related cell lines, we are studying the relation between drug-sensitivity and mutations.

Molecular pharmacology of anticancer agents

We are investigating the pharmacological actions of inhibitors (bromodomain and extra terminal inhibitor 151 [I-BET151] and JQ1) of bromodomain and extraterminal domain proteins. Proteins containing bromodomain connect transcription-related proteins to target genes through binding to acetylated histones. They also recruit disruptor of telomeric silencing-1-like histone H3 methyltransferase (DOT1L1) to facilitate the transcription of RNA-polymerase II. This megaprotein complex forms active transcriptional machinery that regulates genes for cell proliferation and survival. However, the sensitivity of leukemia cells to I-BET151 differs among cell lines. The reason, why only a few cancers are sensitive to I-BET151 remains unclear. To answer this question, we compared messenger-RNA expression patterns before and after treatment with I-BET151 in the most sensitive cell line, JAM911. We found that the genes related to the immune system were markedly down-regulated after treatment with I-BET151. We are now attempting to identify

the gene that is substantially involved in this sensitivity.

Acquisition of drug resistance by cancer cells is inevitable in clinical cancer treatment; we are studying the resistance to I-BET151 in leukemia cells. First, we established U937 cells that are 100 times more resistant to I-BET151 than are parental cells. Using the resistant cells, we are now searching among candidates for genes that are responsible for the resistance.

Molecular genetical approach to neurological diseases

The homozygous deletion and mutation of the survival of motor neuron 1 gene (*SMN1*) causes the hereditary neurodegenerative disorder, Spinal Muscular Atrophy (SMA), which is characterized by progressive loss of alpha-motor neurons in the spinal cord. Recently, we found that the translation of SMN protein is inexplicably regulated by the heterogeneous nuclear ribonucleoproteins which control protein production through several steps. One step is stabilizing the full length of SMN messenger RNA, and another step is enhancing the translation of SMN protein. As a result, SMN protein production is regulated through a highly sophisticated manner, and these findings will provide new avenues for developing drugs to treat SMA.

Alzheimer's disease (AD) is a progressive and incurable degenerative condition. We studied the genetic risk factors of AD, and found that the single-nucleotide polymorphisms of genes for brain-derived neurotrophic factor (*BDNF*) and nerve growth factor (*NGF*) are related to the progression of AD. We are now studying the DNA methylation status of the promoter region of these 2 genes. The *BDNF* promoter seems to be associated with the manifestation and clinical presentation of AD. The DNA methylation of the *BDNF* promoter may be significantly related to the manifestation of AD and might be associated with its neurocognitive presentation.

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Core Research Facilities for Basic Science (Division of Molecular Cell Biology)

Yoshinobu Manome, Professor and Director Takeo Iwamoto, Associate Professor Keiichi Ikeda, Assistant Professor Akito Tsubota, Professor Toshiaki Tachibana, Associate 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).

1. Annual Registration System

This system is intended to supply research space, benches, and other equipment to researchers of the university to perform experiments. Once registered, researchers can freely use the various devices, such as fluorescent microscopes, optical microscopes, and equipment for the preparation of samples for histological examinations, high-performance liquid chromatographs, and nucleic acid amplification systems (polymerase chain reaction). Because inspections and maintenance are regularly performed by the staff, the equipment is reliable and available at any time. This system also provides technical advice and guidance on specific fine morphological or biochemical approaches to a registrant's experiment, if necessary.

2. System for Providing Research Services

Advances in research technologies and equipment enable us to perform more precise and accurate observations of specimens in medical sciences. However, the various new high technologies and devices require specialized knowledge. These advances can cost the researchers both time and money. Also, all researchers are not necessarily familiar with all the equipment for medical experiments. For researchers who cannot perform experiments owing to limits of time and funds, our staff can prepare samples for scanning electron microscopy and transmission electron microscopy, record images, or perform high-performance liquid chromatography and mass spectrometry. By using this system, researchers can proceed efficiently. The service fee is minimal because services are limited to the university.

Research Activities

Drug and molecular delivery by a frog into malignant brain tumor cells

The introduction of foreign materials into cell bodies is an important technique for investigating the functions of corresponding materials. We have previously used ultrasound to transduce such materials. A recently produced mass-producible nanoprinting frog has enabled high-performance drugs or nucleic acids to be transferred into cells. Because little is known about the mechanism of action, we investigated its effects on a malignant glioma cell line. Local oxidation reaction during the nanoprinting by the frog caused reversible cell perforation. The perforation was clearly observed by morphology. The system might be useful for transferring foreign materials into malignant brain tumor cells.

Morphological observation and metabolism of gemcitabine in a cholangiocarcinoma cell line

Cholangiocarcinoma is one of the most intractable malignancies in the human body. The disease was recently highlighted in Japan when cholangiocarcinomas were found to have developed in 11 of 62 persons who had worked in an offset color proof-printing room or in the front room of a printing company for at least 1 year from 1991 through 2006. The incidence suggests that cholangiocarcinoma is a new occupation-related disease. A cholangiocarcinoma cell line, TK, was established at our university. For further research use, morphology and key enzymes, especially for the metabolism of gemcitabine, a drug against malignant chemotherapeutic, were investigated and reported. Since this cell line was well characterized, it may play a role in investigating carcinogenesis and chemotherapy for patients with cholangiocarcinoma.

Transporter genes, single nucleotide polymorphisms, and resistance-associated variants in the treatment of chronic hepatitis C virus infection

Direct-acting antiviral agents (DAAs) or ribavirin or both are the main components of combination treatment for chronic infection with hepatitis C virus (HCV). For the virus to be eradicated with ribavirin-combined treatment, the HCV in hepatocytes must be exposed to ribavirin. Ribavirin is transported into hepatocytes by cell membrane transporters. We have discovered and are investigating the novel function of transporters and the association of single nucleotide polymorphisms of the gene with treatment response. Currently, the role of DAA transporters, causing single nucleotide polymorphisms of DAA-induced liver hepatitis, and resistant-associated variants are being investigated in detail.

Comprehensive gene expression profiling analysis of microRNA/messenger RNA in liver tissue

We are profiling and analyzing the expression of microRNA (miRNA)/messenger RNA (mRNA) in the liver tissue of patients with chronic HCV infection who would undergo combination treatment with PEGylated interferon- α and ribavirin. We have analyzed whether the microRNA/mRNA candidates can be associated with treatment responses in chronic HCV infection. We have found the novel interaction between miRNA and mRNA in replication and lifecycle of HCV. Currently, the expression of miRNA/mRNA in mice that are infected with hepatitis B virus and have humanized liver is being investigated in detail.

Study of the biophysical properties and transfection efficiency for branched amphiphilic peptide capsules to form peptiplexes with DNA

We recently designed a new class of branched amphiphilic peptide capsules (BAPCs) that
self-assemble into extremely stable nanospheres. We attempted to use BAPCs as a carrier of vector plasmids for gene therapy. The BAPCs mixed with genes were delivered to cells without cytotoxicity. However, the transfection efficiency was not high enough to apply gene therapy to mice. We investigated the biophysical properties and transfection efficiency for BAPCs to form peptiplexes with DNA. We found that, in the presence of double-stranded plasmid DNA, BAPCs are unable to be formed. Instead, depending of the peptide/DNA ratios, the peptides either coat the plasmid surface forming nanofibers (high peptide to DNA charged ratio) or condense the plasmid into nanometer-sized compacted structures (at low peptide to DNA charged ratios). Different gene delivery efficiencies are observed for the 2 types of assemblies. The compacted nanometer-sized structures display much higher transfection efficiencies in HeLa cells. This level of transfection is greater than that observed for a lipid-based reagent when the total number of viable transfected cells is taken into account.

Cytopathological analysis of the spinal ganglion in the novel ataxia mouse

Many vacuolar degenerated perikarya were found in the spinal ganglion of an ataxic mouse. No nucleus and endoplasmic reticulum was found in the vacuole, which contained mitochondria, microtubules, and neurofilaments. Each vacuole was encompassed with satellite cells. The satellite cell of a phenotypically normal mouse extended many cellular processes, while that of the ataxic mouse had few processes. These results suggest that the satellite cell is involved in the vacuolar degeneration of the ganglion cell.

Intracellular dynamics of urocortin I in A172 human glioblastoma cells

We recently reported that urocortins (UCNs; UCN I-III) and corticortropin-releasing factor (CRF) receptors are expressed in human gliomas and gastric cancers. However, the secretory pathways of UCNs have not been clearly investigated. Therefore, we investigated the secretory pathway of UCN I in human glioblastoma cells, A172. After construction of a plasmid expressing the hybrid protein of UCN signal peptide and mCherry fluorescent protein (pUCNIS-mCherry plasmid), the plasmid was introduced into A172 human glioblastoma cells. The intracellular dynamics of pUCNIS-mCherry were tracked with a DeltaVision Core-SP fluorescent microscope (GE Healthcare Life Sciences, Marlborough, MA, USA) and several agents. This study suggests that UCN I is secreted through the constitutive pathway.

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

Recent technical innovations has made it possible to mass produce various nano materials. Although nano materials have improved in quality and are being used to produce consumer products, such as foods and cosmetics, their safety is still being investigated. Recently, we have investigated the effect of nano particles on several brain cells. Our results showed < 100 nm nano particles affected the cellular viability and differentiation of neural stem cells. This year, we published these results in the *International Journal of Molecular Sciences* and started a new toxicological study of nano particles against cells related to the blood-brain barrier.

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Laboratory Animal Facilities

Hirotaka Kanuka, Professor and Director

Tatsuya Sakurai, Assistant Professor

General Summary

The purpose of the Laboratory Animal Facilities (LAF) is to support *in-vivo* research and to contribute to the development of basic and clinical medicine. In 2014, about 570 researchers were registered as users of the LAF. 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 lifecycle stage developments of *Trypanosoma congolense*, the cause of animal African trypanosomiasis, are reproducible *in vitro*. Taking advantage of this *in vitro* culture system, we are seeking targets to develop novel methods of controlling this disease. We are now studying molecular mechanisms underlying adhesion of parasite cells to host or tsetse tissues and lifecycle stage developments that are essential biological processes for the parasite to be transmitted. Based on the results of proteome and biochemical analyses, the characterization of molecules predicted to be involved in signal transduction or those expressed on the parasite cell surface is being carried out.

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 eyes 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 diagnostic value of our FOBT method.

Amino acid-related host nutrition dynamics during Plasmodium infection

Malaria, a major parasitic disease affecting more than 200 million people, is caused by the *Plasmodium* parasite. Preventive and therapeutic methods against malaria need to be established because of the expansion of areas suitable for malaria vector mosquitoes with changes in global climate and the emergences of multiple drug-resistant *Plasmodium* strains. *Plasmodium* parasites are 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 the novel interactions between the parasite and host 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. Furthermore, the treatment of mice with food in which the amino acid composition has been modified significantly inhibited parasitemia. Currently, in an *in vitro* model, we are culturing the parasite with human sera from healthy individual donors (more than 3,000 sera are given by the Japanese Red Cross Society) to find aminograms that have an inhibitory effect on the proliferation of *Plasmodium falciparum*.

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Radioisotope Research Facilities

Kunihiko Fukuda, Professor and Director

Yukio Yoshizawa, Assistant Professor

General Summary

The Radioisotope Research Facilities was established to support medical and biological research with radioisotopes. The Facility also accepts research with nonradioactive isotopes. We have supported researchers by suggesting methods and practical techniques for experiments. Lectures and training courses are held for researchers, medical students, and graduate students. In 2014, 35 researchers from 12 departments and 13 students of 2 curriculums used the laboratory of this facility. Major nuclides used for experiments were ³²P, ⁵¹Cr, ¹²⁵I, ³⁵S, 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.

Research Activities

Analysis of resistance mechanisms in radiation-resistant organisms

Tardigrades, which are called water bears, can tolerate extreme environments, including ionizing radiation and dryness. To clarify the radiation-resistant mechanism of the water bear, the sludge water bear *Isohypsibius* isolated from the activated sludge in Ariake Water Reclamation Center were irradiated with X-ray at 450 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 cell preparation step may need to be improved by removing impurity with a cell strainer.

Measuring and tracing of radioactive fallout in the environment

Distribution and behavior of radioactive fallout released into the environment by the accident of the Nuclear Power Plant has been investigated. Recently, since the contaminated water was leaked into the ocean by the accident, we examined a safe, simple, and rapid method of analyzing radioactive strontium in seawater. Radioactive strontium was separated by a solid extraction column of Analig Sr-01 (IBC Advanced Technologies, Inc., American Fork, UT, USA) and was measured with a liquid scintillation counter. With this method the chemical separation of 2 days could be evaluated, compared with 2 weeks with a conventional technique. The detection limit in this procedure from 1 liter of seawater was 1Bq/l. This method might be able to be used to screen 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 concentration in the environment reflects the underground structure. Therefore, repeatedly measuring the radon concentration is useful. We measured radon concentration of 2 famous radon hot springs with a liquid scintillation counter. The radon concentration of Misasa Onsen Ishi-yu (Misasa, Tottori, Japan) was 1920 Bq/l (compare with 1470 Bq/l last year) and that of the Masutomi Onsen Furoukaku Reisen (Hokuto, Yamanashi, Japan) was 1470 Bq/l.

Publications

Minowa H. Image analysis of radiocesium distribution in coniferous trees two years after the Fukushima Daiichi Nuclear Power Plant accident. *J Radioanal Nuc Chem.* 2015; **303:** 1601-5.

Horiuchi K, Minowa H, Yoshizawa Y. 100 Years of radon research of Misasa Hot Springs (in Japanese). Onsen Kagaku. 2015; **64:** 409-21.

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 this facility.

Research Activities

Regenerative medicine for prevention of hearing impairment after the operation for pearl tumor in the ear

Hearing impairment after the surgical treatment for pearl tumor is often induced by the adhesion of auditory ossicles in the ear. To prevent adhesion of auditory ossicles, sheets of autologous nasal cells are to be attached on the surface of auditory ossicles during the operation. Several patients have received this treatment in operations for pearl tumors, and the preventative effect on hearing impairment is now being examined.

Immunotherapy with dendritic cell vaccine against glioblastoma

The combination therapy with temozolomide and dendritic cell vaccine therapy against glioblastoma was performed. Patients with glioblastoma were assigned to 2 groups: a group with recurrent glioblastoma after failing temozolomide chemotherapy or a group with newly diagnosed glioblastoma. Autologous cultured glioma cells obtained from surgical specimens were fused with dendritic cells using polyethylene glycol treatment. Both progression-free survival and overall survival in patients with recurrent glioblastoma (n = 9) or newly diagnosed glioblastoma (n = 20) were significantly longer in patients who received immunotherapy than in control patients who did not.

Combined treatment with Wilms' tumor 1 peptide-pulsed dendritic cells and the anticancer drugs against advanced pancreatic cancer

Combined treatment was performed with dendritic cells pulsed with Wilms' tumor 1 peptide, both HLA classes I and II restricted antigenic peptides, and anticancer drugs, mainly gemcitabine or S-1, against advanced pancreatic cancer. Because a female patient has survived longer than 2 years in good physical condition, her dendritic cells were generated periodically from the peripheral blood mononuclear cells in this facility.

Publications

Koido S, Homma S, Okamoto M, Takakura K, Mori M, Yoshizaki S, Tsukinaga S, Odahara S, Koyama S, Imazu H, Uchiyama K, Kajihara M, Arakawa H, Misawa T, Toyama Y, Yanagisawa S, Ikegami M, Kan S, Hayashi K, Komita H, Kamata Y, Ito M, Ishidao T, Yusa S, Shimo*daira S, Gong J, Sugiyama H, Ohkusa T, Tajiri H.* Treatment with chemotherapy and dendritic cells pulsed with multiple Wilms' tumor gene 1(WT1)-specific MHC class I/II-restricted epitopes for pancreatic cancer. *Clin Cancer Res.* 2014; **20**: 4228-39.

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.

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 developing a 4D human body model that is able to deform several kinds of soft tissue, such as the skin and abdominal organs, skeletal muscle, and vascular system. We are also developing a display system that visualizes rapidly changing childhood growth with X-ray CT data.

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.

This year, we made improvements to increase the movable range of an overtube that has a flexure mechanism to maintain the robot's posture in the abdomen.

We are also continuing our research on a multiview camera system for endoscopic and robotic surgery.

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.

Continuing from last year, we have made progress in integrating surgical navigation systems. We have developed an information display system that enables the surgeon to more easily understand navigation during surgery, which reflects in an operative plan based on a preoperative simulation.

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.

We developed methods to increase the accuracy of navigation during laparoscopic surgery in addition to a navigation function for trocar positioning and conducted clinical applications.

Application of high-definition medical image analysis to forensic medicine

By applying technology that we have developed for analyzing high-definition medical images, we are analyzing X-ray CT data sets of crime victims with the aim of developing new methods for future criminal investigations and for establishing new methods for creating court documents. As we did last year, this year we carried out 3D analyses of the position, depth, and angle of the attempted-murder victim's injuries using the victim's X-ray CT data set.

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

Okamoto T, Onda S, Yasuda J, Yanaga K, Suzuki N, Hattori A. Navigation surgery using an augmented reality for pancreatectomy. *Dig Surg.* 2015; **32:** 117-23.

Onda S, Okamoto T, Kanehira M, Suzuki F, Ito R, Fujioka S, Suzuki N, Hattori A, Yanaga K. Identification of inferior pancreaticoduodenal artery during pancreaticoduodenectomy using augmented reality-based navigation system. J Hepatobiliary Pancreat Sci. 2014; 21: 281-7.

Reviews and Books

Okamoto T, Onda S, Yanaga K, Suzuki N, Hattori A. Clinical application of navigation surgery using augmented reality in the abdominal field. *Surg Today.* 2015; **45:** 397-406. Epub 2014 Jun 6.

Institute of Clinical Medicine and Research

Toya Ohashi, Professor and Director

Takashi Sasaki, Professor

General Summary

Reorganization of the full-time faculty members (researchers) has been significantly performed. On the other hand, a total of 3 research technicians and office employees have been included in the same facility as they were last year. Along with the start of the institute, we have expanded research support of Kashiwa Hospital divisions, including gastroenterology and hepatology, neurosurgery, clinical laboratory medicine, diabetes, metabolism and endocrinology, general medicine, obstetrics and gynecology, and surgery, to help their progress in research themes.

Research Activities

Study of glucose and lipid metabolism abnormality through application with measurement of biological gas component

A specific composition of the biological gas component, such as breath, has been reported when observed in metabolic disorders. For example, breath analysis has been approved by the United States Food and Drug Administration as a noninvasive diagnostic method of a test for rejection occurring after heart transplantation. To apply the same technology for the early noninvasive diagnosis of glucose and lipid metabolism, we analyzed the volatile components of the very small amount of gas of biological origin, including skin gas. In the 2014 fiscal year, we performed basic and clinical studies for this reason. For the analysis, skin gas was collected in a dedicated bag, and the volatile components were adsorbed on a thermal removable collection tube. The volatile components were then quantitatively determined with gas chromatography. As a result, differences in the gas composition from patients with metabolic syndrome and type 2 diabetes mellitus have been observed when compared with healthy control subjects. Specifically, compositions of some substances, e.g., acetone, with strong signals by gas chromatography were changed in association with the disorder. We are planning to develop a new noninvasive method and devices for measuring biomarkers in the near future. This study was performed in collaboration with Division of Diabetes, Metabolism and Endocrinology, Associate Professor Takeo Iwamoto, and Canon Inc.

A study of the injury mechanism and regenerative medicine of pancreatic islets in diabetes

Injuries of cells in endocrine pancreatic islets play a critical role in the development and progression of diabetes. In the 2014 fiscal year, we studied "self-organization of the islets" with Schwann cells covering the entire periphery of the islet based on a completely new concept of "cell protection from metabolic stress." In islet Schwann cells, the same cells as brain astrocytes, regulating the microcirculation of pancreatic islets through the

glutamate transporter should play an important role in protecting against the cell damage caused by oxidative stress through the nuclear factor (erythroid-derived 2)-like 2 pathway. We are planning to study further for the recovery of this metabolic pathway and finally prevention of β -cell dysfunction by using a high-efficiency gene transfer method that we developed in a recent study.

Research cooperation with the research institute of science and technology of the Tokyo University of Science

The Tokyo University of Science and The Jikei University have already conducted education and research cooperation. Because of a geologic advantage, some of research projects had been already performed jointly with the Tokyo University of Science. To promote this relationship, the first joint symposium was held in the 2014 fiscal year. At the symposium with the attendance of Professor Senya Matsufuji, president of The Jikei University, and Professor Yasutaka Moriguchi, vice president of the Tokyo University of Science, 7 original papers were presented and subjected to discussion.

Publications

Seino Y¹, Sasaki T, Fukatsu A², Ubukata M³, Sakai S³, Samukawa Y³ (¹Kansai Electric Power Hosp, ²Yachiyo Hosp, ³Taisho Pharm). Efficacy and safety of luseogliflozin as monotherapy in Japanese patients with type 2 diabetes mellitus: a randomized, double-blind, placebo-controlled, phase 3 study. *Curr Med Res Opin*. 2014; **30**: 1245-55.

Seino Y¹, Sasaki T, Fukatsu A², Sakai S³, Samukawa Y³ (¹Kansai Electric Power Hosp, ²Yachiyo Hosp, ³Taisho Pharm). Efficacy and safety of luseogliflozin monotherapy in Japanese patients with type 2 diabetes mellitus: a 12-week, randomized, placebo-controlled, phase II study. *Curr Med Res Opin.* 2014; **30:** 1219-30.

Seino Y¹, Sasaki T, Fukatsu A², Ubukata M³, Sakai S³, Samukawa Y³ (¹Kansai Electric Power Hosp, ²Yachiyo Hosp, ³Taisho Pharm). Dose-finding study of luseogliflozin in Japanese patients with type 2 diabetes mellitus: a 12-week, randomized, double-blind, placebo-controlled, phase II study. *Curr Med Res Opin*. 2014; **30**: 1231-44.

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 as a stronghold to consolidate the work of research groups on studying pain, whether clinically or biomedically, at 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 inevitably "painful", causes suffering, and is accompanied by strong negative emotions. This latter characteristic also helps individuals remember the potentially harmful situations. However, such emotional aspect of pain also results in decreased quality of life, prevents concentration and rest, and leads the patients to various mental disorders including depression and anxiety, which often leads 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 core mechanism responsible for such clinically "undesired" pain. In particular, chronic pain, of which numerous patients suffer (>15% of the population in major countries), is now thought to be established through changes in the widely distributed neural networks (i.e., neural plasticity) underlying the sensory, cognitive, and affective dimensions of pain. The JCNP aims to integrate the activities of the diverse research teams in The Jikei University and other institutions to establish a strongpoint 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 chronification process of pain (in which pain changes from acute to chronic) and to evaluate the

effects of various therapeutic interventions, such as the transcranial magnetic stimulation. 2. Core for the specific disease-associated pain (core leader, Toya Ohashi)

This core aims to identify mechanisms underlying aberrant specific pain accompanying specific types of diseases, such as Fabry disease, syringomyelia, poststroke pain, postherpes pain, fibromyalgia, and painful diabetic neuropathy. Taking advantage of The Jikei University Hospital, which is visited by many patients with these diseases, this core will use various approaches, including animal models of disease, primary cultured cells, and induced pluripotent stem cells derived from patients, and attempt to translate the findings in the animals to clinical applications.

3. Core for the pain in human patients (core leader, Shoichi Uezono)

This core deals with the pain of multiple etiologies frequently observed in patients. Such pain includes postoperative pain, cancer pain, and neuropathic pain, most of which are resistant to therapy and have unidentified mechanisms. Collaborations between divisions for biomedical sciences in other cores and clinical departments, such as anesthesiology (including the pain clinic), rehabilitation medicine, orthopedic surgery, neurology, and neurosurgery, are promoted in this core. The detailed clinical analyses of the sensory, cognitive, and affective dimensions of pain in relation to other clinical observations in each patient will be used to develop and examine novel strategies against therapy-resistant complications of chronic pain.

Close mutual interactions between these cores will be promoted with the strong leadership of the directors and the Department of Neuroscience, where the headquarter of the JCNP is located.

Health-Care Center

Mikio Zeniya, Professor and Director Masanobu Kaji, Professor Masako Iwanaga, Assistant Professor Takashi Wada, Professor Hiroki Takahashi, Assistant Professor Hiroko Nogi, Assistant Professor

General Summary

Our purpose is to prevent cancer, a lifestyle-related disease, at an early stage through the general health check (referred to in Japan as "*Ningen* Dock"). In 2015, we studied the correlation of hyperuricemia with lifestyle and the detection of fibrosis in patients with nonalcoholic fatty liver disease.

Research Activities

A healthy lifestyle often prevents hyperuricemia. Three simple and easily used healthy lifestyle mottoes have been proposed by Breslow, Morimoto, and Ikeda. We performed a study to determine which of the 3 mottoes is best able to prevent hyperuricemia. The effect of the score of each of the 3 healthy habits on the development of hyperuricemia was assessed with Cox-hazard regression analysis. The cumulative 6-year incidence of hyperuricemia was calculated and compared among the 3 healthy habits groups by means of the log-rank test adjusted for age in the present open retrospective cohort study. The subjects were 5,049 persons who had undergone medical checkups and responded to a self-administered questionnaire about the above healthy lifestyle habits. Hyperuricemia was defined by a serum uric acid level greater than 7.0 mg/dl in men and greater than 5.5 mg/dl in women or by treatment with antihyperuricemia agents during follow-up. For the Breslow and Morimoto habits, hyperuricemia was reduced only in men, but practicing Ikeda's healthy habits significantly reduced the development of hyperuricemia in both men and women.

We investigated the distribution and characteristics of the fibrosis index based on four factors (FIB-4), which indicated liver fibrosis, in Japanese persons who had undergone comprehensive general health checkups and were identified as having nonalcoholic fatty liver disease. Of the 9,255 subjects who had undergone comprehensive general health checkups, 2,750 (29.8%) were found, on the basis of ultrasonographic findings, to have a mildly fatty liver or a fatty liver. After excluding subjects who consumed \geq 150 g alcohol/ week (818 subjects), subjects testing positive for hepatitis B surface antigens or hepatitis C virus antibodies (184 subjects), and subjects for whom data were insufficient (3 subjects), we investigated the FIB-4 indices in the remaining 1,745 subjects. In these patients, the cut-off index was low (< 1.30) in 1370 patients (78.5%), indeterminate (1.30-2.67) in 357 patients (20.5%), and high (> 2.67) in 18 patients (1.0%). The log FIB-4 index differed significantly between subjects with and without a mildly fatty liver or a fatty liver on ultrasonography among men (0.006 ± 0.43 and -0.092 ± 0.39 , p < 0.001), but not among women. The FIB-4 index must be calculated separately during medical checkups and evaluated in conjunction with ultrasonographic findings.

Publications

Torimoto-Sasai Y¹, Igarashi A², Wada T, Ogata Y¹, Yamamoto-Mitani N² (¹Tokyo Med Dent Univ, ²Univ Tokyo). Female family caregivers face a higher risk of hypertension and lowered estimated glomerular filtration rates: a cross-sectional, comparative study. *BMC Public Health*. 2015; **15**: 177.

Onji M, Zeniya M, Yamamoto K, Tsubouchi H. Autoimmune hepatitis: diagnosis and treatment guide in Japan, 2013. *Hepatol Res.* 2014; **44**: 368-70.

Zeniya M, Nakano M, Saeki C, Yokoyama K, Ishikawa T (Saiseikai Niigata Daini Hosp), Takaguchi K (Kagawa Pref Ctr Hosp), Takahashi H. Usefulness of combined application of double-filtration plasmapheresis and twice-daily injections of interferon- β in hemodialysis patients with hepatitis C virus genotype 1b infection and a high viral load. *Hepatol Res.* 2014; **44:** E257-60.

Wada T, Nakano T. Verification of the effectiveness of visual field testing in general medical checkups, particularly in comparison to non-contact intraocular pressure testing. *Sogo Kenshin.* 2014; **41:** 513-7.

Wada T, Zeniya M. Background of the FIB-4 index in Japanese non-alcoholic fatty liver disease. *Intern Med.* 2015; **54:** 127-32.

Wada T, Hasegawa Y^I, Osaki T^I, Ban H^I ('Hitachi Co.). Among three sets of recommended healthy lifestyle habits, which one is most closely associated with prevention of hyperuricemia in Japanese? Ningen Dock International, 2015: **2:** 76-83,

Wada T, Kawasaki Y, Inaji J. Establishing borderline and at-risk regions for estimated skeletal muscle mass of legs determined with a body composition meter. *Ningen Dock International*. 2014; 2: 14-8.

Zeniya M, Wada T. The therapeutic effect of UDCA is a factor in determining the prognosis of primary biliary cirrhosis. *J Gastroenterol.* 2014; **49**: 1438-9.

Clinical Research Support Center

Shigeru Kageyama, Professor and Director

Masako Nishikawa, Associate 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 19 protocols of consultation. Consultations were as follows: 8 protocols for protocol planning and statistics (objective of the research, study design, control arm, randomization, primary endpoint and its rationale, procedure to avoid/reduce bias, data collection, stopping criteria, statistical analysis, analysis sets, methods of handling missing values, sample size calculations, and estimation of study duration), 1 protocol for preparing a random allocation table, and 10 protocols for conducting statistical analysis. Consultations were requested by the Departments of Anesthesiology, Diabetes, Metabolism and Endocrinology, Neurosurgery, Cardiovascular Surgery, Digestive Surgery, Clinical Oncology/Hematology, Endoscopy, and Psychiatry; the IT Strategy Office; 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 survival data analysis, conflicts of interest in medical research, how to prevent misconduct of clinical research, and ethical guidelines for medical and healthcare research involving human subjects.

Research Activities

Statistical methods of analyzing survival 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." We explored the performance and properties of the Lunn-McNeil technique, which enables the statistical inference and testing of differences in the hazard ratio between competing risks for the same risk factor in a single model formulation, with a simulation study. The performance and properties of the Lunn-McNeil technique was compared with those of a separate model, in which the Cox proportional hazards model is applied by a competing risk.

In survival data analysis, we often encounter "partly interval-censored data," for example, progression-free survival in oncology studies, in which observed data include both the exact times of events (e.g., death) and "interval-censored data" (e.g., progression). Among regression methods for partly interval-censored data, we compared the performances and properties of the R package "IntCox," which has been used for interval-censored data analysis recently in R packages, with those of the most common, but ad hoc,

method (to impute a certain value for a censoring interval (e.g., the right-point, the midpoint of the censoring interval), then applying the Cox proportional hazard model) due to the lack of an appropriate method or available software, by simulation study of 200 patients in which the design was based on actual clinical trials.

Publications

Ooba N¹, Sato T², Wakana A³, Orii T⁴, Kitamura M, Kokan A⁵, Kurata H, Shimodozono Y⁶, Matsui K⁷, Yoshida H, Yamaguchi T⁶, Kageyama S, Kubota K¹ (¹Grad Sch Med Univ Tokyo, ²Tokyo Univ Sci, ³MSD K.K., ⁴NTT Med Ctr, ⁵EliLilly Jpn, ⁶Kagoshima Univ, ⁷Showa Univ, ⁸Tohoku Univ Grad Sch Med). A prospective stratified case-cohort study on statins and multiple adverse events in Japan. *PLoS One*. 2014; **9:** e96919.

Kadokura T, Akiyama N¹, Kashiwagi A², Utsuno A¹, Kazuta K¹, Yoshida S¹, Nagase I, Smulders R³, Kageyama S (¹Astellas Pharma *Inc.,* ²*Shiga Univ Med Sci,* ³*Astellas Pharma Global Dev Eur).* Pharmacokinetic and pharmacodynamic study of ipragliflozin in Japanese patients with type 2 diabetes mellitus: a randomized, double-blind, placebo-controlled study. *Diabetes Res Clin Pract.* 2014; **106:** 50-6.

Reviews and Books

Kageyama S. Investigator-initiated clinical trials score and quality assurance (in Japanese). *Iyaku Janaru*. 2015; **51**-5.

Premedical Course

Biology

Koji Takada, Professor

Rie Hiratsuka, Associate Professor

General Summary

Our research themes are the mechanisms of heavy metal toxicity, the abnormal behavior of mice in terms of the activity of ubiquitin-specific peptidase 46, and the mechanism by which the generative cell of angiosperm is taken into a pollen tube cell.

Research Activities

Involvement of protein aggregates in cell toxicity of heavy metals

Toxic heavy metals induce the accumulation of protein aggregates in human kidney HK-2 cells. To verify this phenomenon in other human cells, neuroblastoma SH-SY5Y and hepatoma FLC-4 cell lines were used for a series of experiments. We found that water-insoluble ubiquitin-protein conjugates were also accumulated in these cells by exposure to cadmium and confirmed the occurrence of similar protein aggregates detected with fluorescent immunocytochemistry. For the analysis of these aggregates, a novel extraction method using detergents was developed, and a new batch of monoclonal antibody against polyubiquitin was prepared for the affinity purification.

How ubiquitin-specific peptidase 46 regulates mouse immobile behavior

Ubiquitin-specific peptidase 46 (USP46) is a member of a family of deubiquitinating enzymes that selectively cleave ubiquitin or ubiquitin chains from target proteins and stabilize them or modify their functions. Previous studies have shown that USP46 is involved in the immobile behavior that allows for retraction from unavoidable stresses and is implicated in the regulation of the GABAergic system, but the precise function is still unknown. We have identified WD repeat domain 48 (WDR48) and dystrophia myotonica WD repeat-containing protein (DMWD) as the target candidates. These proteins bound equally well to the wild-type and mutant USP46, indicating that the deletion mutation of lysine 92 does not affect the interaction of USP46 with the WD repeat proteins.

Mechanism of engulfment of generative cell by pollen tube cell: Observation with highpressure freeze fixation method

The purpose of this study was to clarify the mechanism by which the generative cell (GC) of angiosperm is taken into a pollen tube cell. In this study, high-pressure freeze fixation was used during tissue fixation. As a result, a cell membrane and microtubular preservation were improved, and the following items were clarified. (1) The GC gradually comes off after division from the pollen wall; it changes its dome form to a sphere form and gets projected in a pollen tube cell. (2) In the spherical GC, a large number of microtubules

are dispatched to a cell membrane from a nucleus radially. (3) The cell membrane of the pollen tube cell is tucked inside along the bottom of the dome-formed GC and separates the GC.

Publications

Matsumoto M, Matsuura T, Aoki K, Maehashi H, Iwamoto T, Ohkawa K, Yoshida K, Yanaga K, Takada K. An efficient system for secretory production of fibrinogen using a hepatocellular carcinoma cell line. *Hepatol Res.* 2015; **45**: 315-25. Matsumoto A, Ishibashi Y, Urashima M, Omura N, Nakada K, Nishikawa K, Shida A, Takada K, Kashiwagi H, Yanaga K. High UBCH10 protein expression as a marker of poor prognosis in esophageal squamous cell carcinoma. Anticancer Res. 2014; **34**: 955-61.

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 kingfisher and the red-flanked bluetail, is a structural color owing to the interference of the light within a barb by reproducing the reflection spectrum.

2. We have investigated the influence 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. Phase transitions, critical phenomena, interacting many-body systems, computer simulation

Research Activities

Numerical study on the structural color of blue birds

The article "Numerical study on the structural color of blue birds by a disordered porous photonic crystal model" by Ueta etal has been selected by the editors of EPL for inclusion in the exclusive "Highlights of 2014" collection. It has been observationally confirmed that the color of some birds, such as the kingfisher and the red-franked bluetail, is a structural color owing to the interference of light within a sponge structure inside a barb. In this study, we considered the air rod photonic crystal to which disorder is introduced into the translation vectors and the radius as a model of the structural color of the red-franked bluetail; the optical property of the model was numerically analyzed and compared with that of the structural color.

Enhancement of the dynamic Casimir effect within a metallic photonic crystal by dissipation

The influence of the optical absorption of metallic materials on radiation in the excited states from a vibrating metallic photonic crystal has been investigated. We found that the influence of the optical absorption depends on the mode of radiation and on the form of wave functions and that the optical absorption surprisingly even enhances the output in the excited states.

Monte Carlo simulation of the ferromagnetic Potts models

We have made Monte Carlo simulations to study systems with the first-order phase transition. Multigrid cluster update simulations are used to study q = 4,10 ferromagnetic Potts models on square lattices. We calculated the relaxation times of energy and magnetization.

Publications

Ueta T, Fujii G¹, **Morimoto G**², **Miyamoto K**³, **Kosaku A**³, **Kuriyama T**⁴, **Hariyama T**⁵ (¹Shin**shu Univ**, ²**Rikkyo Univ**, ³**Dokkyo Med Univ**, ⁴**Univ Tokyo**, ⁵**Hamamatsu Univ Sch Med**). Numerical study on the structural color of blue birds by a disordered porous photonic crystal model. *Europhys Lett*. 2014; **107:** 34004.

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Fujii G¹, Ueta T, Mizuno M¹ (¹Shinshu Univ).

Level-set-based topology optimization for antireection surface. *Appl Phys A Mater Sci Process*. 2014; **116**: 921-7.

Ueta T, Otani Y¹, Nishimura N¹ (¹Kyoto Univ). Photonic-crystal like approach to structural color of the earthworm epidermis. *Forma*. 2014; **29** (Special Issue): S23-8.

Itoga H, Morikawa R, Miyakawa T, Yamada H, Natsume Y, Ueta T, Takasu M. Shape deformation of vesicles containing hard spheres. JPS Conference Proceedings. 2015; **5**: 011002.

Chemistry

Takashi Okano, Professor

Chikao Hashimoto, 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; the development of new methods for peptide synthesis; and the computer-assisted analysis of materials and synthetic reactions.

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 galactosyl benzyl glycoside, $16,17^{-13}C_2$ -retinol, and $1,4^{-13}C_2$ -putrescine. As the new synthetic tools, an automatic liquid-phase synthesizer and an automatic preparative liquid chromatograph were fully utilized.

Synthesis of N-protected peptide acids using amino acid-alkaline earth metal salts

The protection of a carboxyl group by a metal ion saves the time needed for the incorporation and removal of the protecting group and prevents side reactions caused by the use of esters. The syntheses of N-protected peptide acids in organic solvents using alkaline earth metal-carboxylate salts of an amino acid were investigated. We found that the amino acid-Ca carboxylate salt is the most effective of the carboxylate salts of the amino acids tested for coupling with Boc-amino acid active esters in an organic solvent, such as N,N,-dimethylformamide and dimethylsulfoxide.

Social Science (Law)

Ryuichi Ozawa, Professor

General Summary

Problems of constitutional law in present-day Japan

Research Activities

Ozawa published the following articles and books from research activities in 2014.

Publications

Ozawa R. On the collective self-defense right (in Japanese). *Hogakukan Kenpokenkyujoho*. 2014; **11**: 1-24.

Ozawa R. On new trend of the collective selfdefense right (in Japanese). *Hogaku Seminar*. 2014; **231:** 96-119.

Ozawa R. Sustainable financial system and constitutional reform (in Japanese). *Ho no Kagaku*. 2014; **45:** 78-83.

Ozawa R. On the future of peace in East-Asia (in Japanese). *Horitsu Jiho*. 2014; **Suppl**: 240-5.

Reviews and Books

Ozawa R. On pursuit of peace by law (in Japanese). In: Kido E, editor. Heiwa kenkyu nyumon. Osaka: Osaka University Press; 2014. p. 207-19. **Ozawa R.** Prologue, Chapter 1, Chapter 2 (in Japanese). In: Ozawa R, Sakakibara H, editors. Abe Seiken to jichitai. Tokyo: Jichitai Kenkyusha; 2014. p. 3-7, 19-26, 43-64.

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 Donald 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.

Publications

Misaki K. "Zeitkern" and "Erzählung" — Walter Benjamin on history/memory (in Japanese).

Tetsugaku to Gendai. 2014; 29: 24-45.

Japanese

Ikuko Noro, Professor

General Summary

1) A study of patients' perceptions of shared decision-making and the association with patient satisfaction in the case of chemotherapy

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

Research Activities

A study of patient' perceptions of shared decision-making and the association with patient satisfaction in the case of chemotherapy

On the basis of research regarding patients with cancer, Noro et al. (2015) reported that it was not the kind of decision-making process (shared, patient-centered, physician-centered) that affected patients' satisfaction, but the concordance between the actual decision-making process and the process patients had preferred.

Publications

Noro I, Ishizaki M, Kobayashi R. Patients' perceptions of shared decision-making and the association with patient satisfaction in the case of chemotherapy (in Japanese). Research Survey Reports in Information Studies, Interfaculty Initiative in Information Studies, The University of Tokyo. 2015; **31:** 89-113.

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 the Conley index theory.

2. One of the main topics of our research has been the explicit construction of automorphic forms. Because the representation theory of Lie algebra has played a fundamental role in the theory of automorphic forms, we showed the algebraic structure of the representation and thereby obtained automorphic forms.

Publications

Hasegawa Y. Fourier expansion of minimal parabolic Eisenstein series. Suron Josei no Atsumari

Hokokushu. 2014; 7: 85-91.

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 to investigate 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 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 the textbooks, Fujii has been writing their exercise materials and teacher's manuals. In addition, he has published a conversational English textbook for self-study.

Research Activities

Ohara presented a paper at a session in the International Medieval Congress 2014 held at the University of Leeds in the United Kingdom.

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. Officially approved by the Ministry, the third textbook in the series, *World Trek-English Communication III*, and its instructional aids, *World Trek-English Communication III Teacher's Book* and *World Trek-English Communication III Teacher's Manual*, were published. Fujii also published a self-study English textbook, *Heartfelt English Conversation*, from Kirihara Publishing in Tokyo.

Reviews and Books

Mochizuki M (Reitaku Univ), Aizawa K (Tokyo Denki Univ), Allum P (Rikkyo Univ), Sasabe N (Toritsu Aoyama High), Hayashi Y (Souka High), Fujii T, Miura S (Tsurubunka Univ). World treck English communication III Teacher's Book. Tokyo: Kirihara Publishing; 2015. Mochizuki M (Reitaku Univ), Aizawa K (Tokyo Denki Univ), Allum P (Rikkyo Univ), Sasabe N (Toritsu Aoyama High), Hayashi Y (Souka High), Fujii T, Miura S (Tsurubunka Univ). World treck English communication III. Tokyo: Kirihara Publishing; 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. I had done research on his discourse about India and Africa and the cultural background of this discourse. I have already published the results. I continue researching his discourse about Arabia and studying Arabic culture. In addition to this work, I am translating a book by Johann Ludwig Burckhart, who introduced the Islamic world to the people of Europe in the early 19th century. In addition, on the occasion of the 230th anniversary of his birth I wrote an essay about him.

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 Job Satisfaction and the Work Environment of Certified Nurse Specialists Working in Hospitals. In addition she organaized the 19th Research Conference of Japan Society of Nursing Economics and Policies.

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.

Reviews and Books

Tanaka S. Nurse's life and work (in Japanese). In: Nihon Kango Rekishi Gakkai, editor. Nihon no kango no ayumi. Tokyo: Nihonkangokyokai shuppannkai; 2014. p. 27-42.

Nursing Administration

Midori Nagano, Professor

General Summary

Two studies have been performed: "The characteristics and care needs of ostomates" and "Health work environment in nursing practice"

Research Activities

The characteristics and care needs of ostomates

The aim of this study was to describe the characteristics and care needs of ostomates. The medical records of outpatients with stomas undergoing twice-weekly stoma care at an 800-bed hospital were examined. Ostomates who had used outpatient stoma services after formation of a stoma for rectal cancer from January 2008 through July 2014 were surveyed regarding their treatment visit status.

The 101 ostomates were 69 men and 32 women aged 33 to 93 years with a median age of 64 years. Elderly patients and patients living in 1- or 2-person households require support and planned care, such as outpatient stoma services and home-visit nursing.

We must act to develop local home-visit nursing provision systems and outpatient nursing at hospitals in anticipation of an aging society and increases in single-person households and households that require elder-to-elder nursing care.

Health work environment in nursing practice

We examined earlier documents of the health work environment in nursing practice. Joint research with concerned parties to analyze and develop the health work environment in nursing practice in Japanese hospitals was performed with a questionnaire survey. We analyzed the inventory survey result and describe influence of stress and motivation on nursing administrators. We used the 6-item Kessler Psychological Distress Scale as an index of psychological distress and examined the health of the nursing administrator, difficult feelings, and feelings of worthwhile management. The importance of consultation with the chief manager became clear for an association to health between difficult feelings and feelings of worthwhile management of the nursing administrator and the chief manager.

Publications

Nagano M, Ogata Y, Tokunaga K, Ishikubo Y, Ishida Y. Effective pressure ulcer measures at hospital as determined from certified wound, ostomy, and continence nurses' perception of influence on quality control and the incidence of pressure ulcers (in Japanese). *Nihon Sosho Ostomy Shikkin Kanri Gakkaishi*. 2014; **18:** 293-304.

Adult Nursing

Naomi Takashima, Professor Ruka Seyama, Associate Professor Ako Terakado, Assistant Professor Mai Hosokawa, Assistant Professor Masami Sato, Professor Hiroaki Murata, Assistant Professor Mariko Nakano, 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 an emphasis on the process of learning practical nursing skills through the 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 patients with acute or critical illnesses. Based on the educational evaluation of the past, the new curriculum was established in the 2012 fiscal year and was offered during the year of "adult nursing practice theory" for the purpose of building critical thinking competency. The course content and the deployment methods, for a group learning the convalescence by nursing of surgery patients during the acute phase, were the deployment of the nursing process while incorporating problem-based learning elements and exercise. As a result, in the class evaluation by students the scores were: teaching method, 3.73; course content, 3.40; group work, 3.75; their learning attitude, 3.33. In addition, a shared learning effect was achieved by holding a summarizing practicum conference on the final day. While student evaluations of nursing process development, which included information collection and nursing practice utilizing nursing plans, were largely positive for the overall adult nursing practicum, those of faculty members tended to be lower. In the practicum environment and organizational arrangements, cooperation with clinical practicum instructors was strengthened by setting up opportunities for students to review the work with their instructions. Students generally had positive evaluations of educational interventions by the faculty members, such as faculty being present at the clinical scene and providing advice and critique in a timely manner, providing clues on nursing process development based on records, conducting nursing practice together with the students, and ensuring safety. These are aspects that we hope to continue, and practicum training with appropriate interactions is also anticipated in the future by adjusting the division of roles of the parties involved.

Research Activities

Research on Cancer Nursing

1. Research on nursing care for patients with rectal cancer undergoing anterior resection We have been developing methods of nursing to reduce the bowel disorders typical of anterior resection. The analyses and studies that examine utilizing nursing practice developed were confirmed this year to have a certain effect. In clinical practice, nursing assistance can be expected to collaborate with physicians. Activities including collaboration with the wound, ostomy, and continence nurse based on and discuss with the physician, were introduced academic conference. That will create further improvements and verified the effects of the nursing support program.

2. Research on support for cancer patients undergoing outpatient radiation therapy and their family members

We are developing support for the psychosocial distress of patients who have cancer and are undergoing outpatient radiation therapy and for their family members. This year, we performed interviews with clinicians, were focused the direction of intervention for the patient and family together with the results of a literature review. As a result, it became possible to carry out interventions to populations for limiting the age and functional level. 3. Research on the chemotherapy-induced peripheral neuropathy

In joint research with other facilities, we have been developing an assessment tool for chemotherapy-induced peripheral neuropathy. This year, we have completed the investigation for scale development, which started from scratch last year for the number of samples met the 310 persons. In the future, we are planning to perform statistical processing to determine the scale for measuring the extent peripheral neuropathy factor. In addition, we are scheduled to begin a study support program.

Research on critical care

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

2. The development of the nursing support program for patients with undergoing ventilation

We studied the experience of patients receiving ventilatory support for acute respiratory failure and continued research for the development of a nursing support program. The present study clarified the experiences and coping of patients undergoing noninvasive positive-pressure ventilation. We are planning to evaluate the effect of the development program on intervention.

3. Process of nursing practical in physical restraint by experienced nurses in the critical field

We are searching for the structure of the development of the clinical "knowledge" of nursing students, novices, and expert nurses for the purpose of visualizing nurse clinical "knowledge" innermost feelings in nursing practice called physical restraint.

Characteristics observed in nursing practices by experienced nurses included their deep understanding of patients and their nursing policy that always aims to remove physical restraint. Although experienced nurses were driven by a firm conviction of saving patients' lives, they also felt a conflict in selecting safety or comfort for patients and protect from incidents.

Gerontological Nursing

Miyoko Sakurai, Professor

Junko Kusachi, Associate Professor

General Summary

We investigated strategies for acquiring the necessary knowledge from lectures and seminars that teach nursing techniques, particularly nursing skills for patients using wheelchairs and having pressure ulcers. Methods of training nursing students have changed in the revised 2012 curriculum; therefore, we explore new methods of teaching and evaluation.

Research Activities

Research activities in our field that we have been engaged in are as follows:

1. The results of a study entitled "The development of exercise programs that support the main activities of the elderly requiring care: The effect of stretching the lower limb triceps on lower limb edema relief" were presented at the 34th Academic Conference of the Japan Academy of Nursing Science. Furthermore, continuing our quest for broadening knowledge in related domains, we are working on a study entitled "Comparison of the effects of cushion selection on sitting posture, lower limb edema, and the blood stream during sitting posture assistance in the elderly."

2. The results of a study entitled "Investigation of basic nursing education content, 'the technology to promote bedsore prevention and healing'" were presented at the 40th annual meeting of the Japan Society of Nursing Research.

Mental Health and Psychiatric Nursing

Takeshi Katsuki, Professor

Junko Ishikawa, Assistant Professor

General Summary

The lecture is aimed to teach a medical system based on the Mental Health and Welfare Act and to utilize social resources in the area. The concrete purpose is to investigate major methods of assessments to support patients with mental problems in the treatment process.

Research Activities

We have continued to investigate the mental effects of the Great East Japan Earthquake 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 2014. 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, imaging a great deal of damage in the future, and age more than 60 years with a lack of physical ability.

Thus, we presented an interim report at the 16th Pacific Rim College of Psychiatrists Scientific Meeting 2014 on October 5, 2014.

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

Child Nursing

Kiyo Hamanaka, Professor

Kinu Takahashi, Associate Professor

General Summary

The following studies were performed. For the first study (Study I), it was significant that a paper was published in a medical journal and its results were shared among teaching staff in different colleges. Analytic results from the second study (Study II) were presented at an academic meeting and its conclusions were broadened by a likely discussion with participants. Further verification will be performed on the basis of the findings of the third study (Study III), and then an evaluation table will be prepared to strengthen the developed process.

Research Activities

Study I: Interrelation between advocacy of children's rights and experience of ethics education in nursing

In this study, a questionnaire survey was conducted with 295 pediatric nurses in 8 healthcare facilities located in the Kanto region, including university hospitals, specialized children's hospitals, and general hospitals, to clarify the present status of children's rights advocacy in nursing in connection with nurses' experience with ethics education. The results revealed that 70% of the nurses surveyed had undergone ethics education. However, half of these nurses felt that the content of the education was not well defined. Furthermore, 40% of the nurses who had undergone ethics training reported regularly advocating for children's rights, while 60% practiced such advocacy only some of the time. Concerning actions taken when children's rights were being violated, nurses with less experience in pediatric nursing were less likely to employ ethical measures in such situations. There was a tendency for nurses in general hospitals to be less active in advocating for children's rights than were nurses in university hospitals or specialized children's hospitals. In addition, a qualitative analysis was performed in this study. The reasons for not applying ethics in practice given by less experienced pediatric nurses were considered an issue, both in basic ethics education and on-the-job training. These nurses frequently attributed their behavior to their being new nurses, to a specific problem in the children's wards in university and general hospitals, and to being due to the influence of Japanese culture.

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

Self-administered questionnaires were given to the teaching staff of 214 educational institutions across the country to clarify the current situation of nursing ethics and advocacy of children's rights in basic nursing education.

Results showed that nursing ethics education was offered in 90% of the educational institutions. In 48% of the institutions, 1 or 2 lectures in "Introduction to Nursing Science" courses covered this topic, and in 12% of the institutions, there was a teaching unit on nursing ethics. Furthermore, 44% and 41% of the respondents considered that the current provision of nursing ethics education was adequate and inadequate, respectively. All institutions provided education on the advocacy of children's rights as a separate part of courses, such as "Introduction to Pediatric Nursing," "Methods in Pediatric Nursing," and "Practice in Pediatric Nursing." However, the contents of the "Methods and Practice" courses were found to be inferior to those of the "Introduction" courses. Education on children's rights tended to be perceived as satisfactory in colleges and junior colleges but not in nursing schools. In the future, education methods will be further investigated.

Study III: *The process of pediatric nurses to implement practices that advocate children's rights*

This inductive qualitative study aimed to clarify the processes necessary for pediatric nurses to implement practices that advocate children's rights. The participants were 14 nurses with more than 5 years of pediatric nursing experience, from 3 facilities at university hospitals located in the Kanto region in Japan.

The findings revealed that the core category in this process is "being able to consider children centrally." This category comprised 4 levels, in the following order: "one can act as instructed but cannot consider things independently," "one obeys 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." Furthermore, 3 abilities were found to affect the intensity of this developed process: "confirmation of children's power," "contrivance to convey children's power," and "attractive emotion to children." This process can help to accelerate the practice of pediatric nurses to advocate children's rights in clinical settings involving children, basic nursing education, on-the-job training, and research in colleges.

Study IV: Support and demand for correspondence, surroundings, medical treatment, and care in a hospital as envisioned by parents of children with mild developmental disabilities

An interview survey aimed to clarify demands for correspondence, surroundings, medical treatment, and hospital care, as envisioned by parents of children with mild developmental disabilities. The participants are parents who have experienced the hospitalization of a child. This study is now in progress.

Publications

Takahashi K, Hamanaka K. Interrelation between advocacy of children's rights and experi-

ence of ethics education in nursing (in Japanese). *Herususaiensu Kenkyu*. 2014; **18:** 21-31.

Maternity Nursing

Kimiko Kayashima, Professor

Yasuko Hososaka, Associate Professor

General Summary

Studies have been performed to examine 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

Factors influencing women's resumption of sexual intercourse 4 to 5 months postpartum This study elucidates the factors influencing the recovery of female sexual function in the puerperal period. It also obtains new insights for postpartum sex education to make specific recommendations that are designed to aid a postpartum woman and her partner to comfortably resume sexual relations. The study gathered responses from 166 women (38.2% return rate), of whom 152 gave valid responses (91.6% effective response rate). The mean age was 32.25 ± 4.67 years. Sexual intercourse was resumed by 82 respondents (53.9%) and was not resumed by 70 (46.1%). The mean time for resumption of sexual intercourse was 10.26 ± 5.09 weeks. Women who resumed sex 4 to 5 months after giving birth were likely to be young, to have resumed menstruation, and to have had more frequent sexual intercourse before and during pregnancy.

A descriptive study of the boundary between discipline and abuse

This study aims to identify and describe what sort of childrearing behaviors that mothers who raise preschool children regard as being fair discipline or abuse, to shed light on childrearing behaviors that cause anxiety about raising children, to make them widely known, and to gain insights for assisting such mothers. The study consists of 2 parts. Part 1, "Childcare experiences as related by mothers raising preschool children," is now in progress. We conducted semistructured interviews with 26 mothers, and, from 3 different perspectives, identified 7, 12, and 6 categories, respectively. The findings are presently being analyzed with the Modified Grounded Theory Approach.

Establishment of neonatal cleanliness care: From the smell of neonates and a bacteriological survey

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.

Issues surrounding the placement of maternal transport coordinators and the development of educational programs

Maternal transport coordinators have been established in 14 prefectures in Japan. A survey of coordinators, ambulance crews, and obstetricians was conducted in these regions. The information considered most important for emergency maternal transport differed among the different professions. However, problems related to pregnant women without antenatal care were cited by all the professions as factors making perinatal transport difficult and as issues to be addressed. Roles and capabilities required of coordinators included knowledge of perinatal medicine, the ability to make prediction-based decisions, liaison communication skills, and negotiation skills. This survey findings indicated the need for training and learning opportunities in these fields.

Publications

Hososaka Y. Construction of a weight control model for normal weight and underweight pregnant women for the prevention of low birth weight infants (in Japanese). *Bosei Eisei*. 2014; **55:** 360-8.

Reviews and Books

Kayashima K. Support of postpartum sexual health (in Japanese). In: Saito M, editor. Guidebook for sexual health and consultation. Tokyo: Chuo Hoki; 2014. p. 152-3.

Community Health Nursing

Junko Shimasawa, Professor Nobuyo Ueda, Assistant Professor Yoshiko Kubo, 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) research on educational evaluation, 3) exploring career anchors among occupational health nurses, and 4) reflection among newly appointed public health nurses in community nursing.

Research Activities

Visiting nursing care for mentally ill patients living at home

The purpose of this study was to clarify 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 individual in a manner suitable for that individual.

Exploring career anchor among occupational health nurses

The concept of "career anchor" is defined as the single element in a person's self-concept that he or she will not give up, even in the face of difficult choices (Schein, 1990). This qualitative interview study aimed to explore the characteristics of career anchors among occupational health nurses in Japan.

Characteristic reflections of new public health nurses in their second to fourth year of employment

The purpose of this study was to identify the reflections characteristic of new public health nurses in their second to fourth years of employment. The characteristic reflections were found to be the novel directions of support, realizations, and results of repeatedly thinking over the events and accompanying emotions that had troubled them in their nursing practice.

Nursing Care for Directly Observed Treatment, Short-Course

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

Publications

Kubo Y, Shimasawa J, Kita M, Takashima N, Takahashi K, Satake S, Hamanaka K, Sakurai M. How is portfolio method effective on supporting

active learning attitude among nursing students? (in Japanese) *Tokyo Jikeikai Ika Daigaku Zasshi*. 2014; **129:** 119-27.
Home Care Nursing

Motoko Kita, Professor Hiroko Toyama, Assistant Professor Reiko Yoshida, 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

Evaluation of the flipped classroom in home care nursing

To help students develop assessment skills unique to home care nursing, preclass learning with e-learning was conducted regarding the mechanisms of diseases and symptoms which were conventionally taught during lectures, and the flipped classroom, in which assessment techniques are mainly taught during lectures, was introduced. An examination of the effects of the flipped classroom observed no difference based on students' level of understanding of assessment techniques, which is a class objective However, student learning during the class was increased and showed that preclass learning with animation contents was beneficial for acquiring basic knowledge. Furthermore, more and more people use their smartphones as a tool to play animation contents, suggesting that they are an effective means to study within a limited time regardless of the place and computer accessibility.

Establishment of the discharge planning process for elderly patients with dementia in acute care hospitals

Recently, a growing number of elderly patients with dementia are being transferred to acute care hospitals for the treatment of other diseases; however, their discharge planning involves difficulties. For the first stage to develop a discharge planning model that can deal with the difficulties of people with dementia, we have been conducting research to identify the discharge planning process for elderly patients with dementia in which nurses of acute care hospitals are involved. We launched a survey this year and conducted an interview involving nurses working in acute care hospitals.

The status of information-sharing with family caregivers perceived by visiting nurses

In home-based care settings, sharing information with family members is important to provide patients with appropriate care. We performed research to clarify patients' information that visiting nurses wish to collect from family members and to clarify the current status of information sharing. The results revealed that patients' information was more

likely to be provided by only nurses. For visiting nurses, understanding patients' health status when nurses are not at their home is extremely important for assessing patients correctly and for predicting health changes that may occur in the future; this finding suggests that a system should be developed to share information with family members with regard to their cohabitation status (living together or separately).

Learning needs of the staff of a comprehensive community support center who provide care for the elderly with mild depression

We conducted a literature review to summarize and identify learning needs to empower the staff of a comprehensive community support center who are involved in the care of the elderly with mild depression, as a part of a care prevention program conducted by the center. To develop an effective learning program, we are planning to conduct questionnaire surveys and interviews involving the staff in the future.

Inspection/evaluation

Home Care Nursing has proactively introduced active learning into the class. As a part of the continuous assessment of education, the flipped classroom was evaluated regarding its efficacy in this study. The results suggest that further class improvements are needed. We will continue our educational assessment to offer more effective education.

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.

Index

$1,4^{-13}C_2$ -putrescine ·····	234
¹³ C-labeled galactosyl benzyl glycoside	234
¹³ C-labeled materials	234
$16,17^{-13}C_2$ -retinol ······	234
3D CT	•98

A

accreditation ······2
Achilles tendon ······ 105
acquired immunity181
actin12
active infectious endocarditis119
acute kidney injury143
acute lung injury69
acute respitratory distress syndrome
adeno-associated virus
advanced pancreatic cancer
adverse transfusion reaction171
aerospace medicine175
African trypanosome ·····216
aging of eggs ······ 124
Alzheimer's disease
amino acid-alkaline earth metal salts 234
amino-cupric-silver method177
aminogram ····································
amplifying properties233
amygdala ·····199
amyotrophic lateral sclerosis ······206
angiogenesis 58
angiopoietin ·······79
ankle sprain ······ 179
annual registration system ······212
antenatal stress ······124
antibody therapy191
anticancer chemotherapy
antiepileptic drugs75
anti-PEG IgM antibldy196
antizyme ······22
antizyme inhibitor
aortic valve replacement119
apoptosis ······69
arrhythmia ······60
arsenic ······43
artficil protein vaccine ·····191
arthroscopic reduction and internal fixation 179
articular disk ······ 167
artificial ventilatory therapy143

aspiration pneumonia ······146
astaxanthin ······173
astrocyte 4, 199
astronauts ······ 175
atopic dermatitis ······83
ATP citrate lyase ······22
atrial fibrillation ······60
atypical polypoid adenomyomas29
autofluorescence imaging155
autofluorescent endoscopy48
autoimmune hepatitis ······29, 48
automorphic forms ······237
autophagy
axonal transport ······206

B

Burckhardt, Johann Ludwig ······239

С

Ca ²⁺ transient	15
cadmium ·····	
cancer 43,	216, 242
cancer cells	
capsule endoscopy	155
cardiac fibrosis ·····	15
cardiomyopathy	15, 60
cardiopulmonary	119
carrier support	2
case-cohort design	201
cell adhesion	
cell cycle ·····	
cell differentiation	
cell fusion ·····	220
cell sheet ·····	····· · 220
cementless femoral reconstruction	
central nervous system ······	4
cerebellar degeneration	
cerebro-cerebellar communication	
certified nuse	
CGM	
chain recurrent ······	
check-back ······	
chemoimmunotherapy	
chemotherapy	
child abuse	
child nursing	
child-rearing anxiety	
children with incurable diseases	····· · 245
children's health promotion	
children's rights	
chlorogenic acid ······	
cholesteatoma	139 220
chromic obstructive pulmonary disease ·	····· 69
chronic care model	
chronic hepatitis B	
chronic hepatitis C	
chronic kidney disease	
chronic obstructive pulmonary disease	105
chronic pain	
citrullination	
clinical decision making	
clinical epidemiology	
clinical ethics	
clinical pharmacology	
clinical research	203 230
······································	200, 200

clinical research coordinator	···201
clinical study	64
clinical trial	230
Clostridium difficile	163
c-MYC ·····	22
cognitive-behavioral therapy	75
cohort study ······	204
collagen-induced arthritis	58
colon capsule endoscopy	155
color vision	131
colorectal neuroendocrine tumor	29
colorectal serrated lesion	29
comfort care ·····	242
Common Marmoset ·····	206
communication	236
community health nursing practice	···· 249
complexity	204
complication	
computational fluid dynamics	111
conditioned fear response	199
congenital diaphragmatic hernia	9
constitution	235
constraint-induced movement therapy	146
contact dermatitis/ drug eruption	83
continuous ambulatory peritoneal dialysis ·	182
continuous theta burst stimulation	146
coordinator	···· 247
copy number variants	203
cortical vessel signs	51
craniofacial anomaly	···· 111
critical care ·····	···· 242
critical period	•••••• 4
Crohn's disease	
cryoablation	
cryotherapy	129
CT-based navigation	105
curriculum ·····	···· 240
cytotoxic T lymphocytes	194

D

DAT SPECT 51
databank ······149
decompression stress ······43
deep venous thrombosis
dementia ······250
democracy ······235
dendritic cell ······ 220
depressive disorder
dermatomyositis

development	•••• 4, 9
developmental disorders	75
diabetes ·····	64
diabetes mellitus	224
diaphragm ······	9
dietary restriction	173
digital medievalism ······	238
dimension theory	237
directly observed treatment short course	···· 249
DNA damage ·····	18
DNA methylation	210
DNA polymorphism ······	46
dog	216
donor chimerism ······	····· 79
dopamine ·····	25
dorsal root ganglia ······	194
drug delivery system ·····	131
drug resistance	163
drug-eluting peripheral stent	102
DSC ·····	12
DTI	··· 206
ductus arteriosus ·····	15
duodenal epithelial tumor	29
dupuytren contracture ······	105
dynamic Casimir effect	233
DYRK2 1	8, 124
dysphagia ·····	···· 244

E

EARTH Study ······ 1	82
ectodermal dysplasia ······ 1	77
edema ······2	.44
elastic fiber ·····	15
elderly patient	73
e-learning ······2, 2	50
electromyography ······1	75
electrophysiology ······4,	60
eltrombopag for ITP	93
emotion ······2	26
ENA-78/CXCL5 ·····	58
endoclinology	64
endoscopic sinus surgery1	39
endoscopic submucosal dissection1	55
endoscopic surgical robot system2	22
endoscopic ultrasound-guided fine needle	
aspiration biopsy ······1	55
endovascular repair ······ 1	02
endozepine ·····	25
English Communication ······2	38

English learning material ······238
enteroscopy ······ 155
enucleations of schwannomas105
enzyme replacement79
enzyme replacement therapy187
eosinophilic chronic rhinosinusitis ······ 88
EPA/AA151
epidemiology ······64
epidermal growth factor receptor139
epigenetic ······210
epilepsy ·····151
epithelial-mesenchymal transition93
epithilial ovarian cancer ······124
eRNA
esophagogastric neoplasia ······155
esophagogastric varices155
evidence-based clinical practice
exercise training173
exome sequencing79
extended-spectrum β -lactamase $\cdots 163$

F

family caregivers250
fasciitis ······58
fatigue ······34
FEATHER Study 182
febuxostat ······182
fecal occult blood ······216
female sexual function247
fertility preservention care ······124
fibromyalgia ······ 226
fin9
fine morphology ······212
fission products ······218
flatfoot
flipped classroom ······250
fluoride43
fontan procedure ·····119
food allergy ······ 203
food factors43
forensic pathology46
forensic toxicology ······46
four dimensional human model ······ 222
fracture of the lateral process of the talus 179
frameshift ······22
Frankl, Victor Emil ······236
Frank-Starling mechanism15
Fukushima Dai-ichi Nuclear Power Plant ····· 218
full-thickness rotator tears105

functional magnetic resonance imaging 131 functional magnetic stimulation 146

G

GABA ···································
gas chromatography ······224
gastric emptying study by ¹³ C-breath test93
gene analysis ······187
gene therapy
general practitioner
generative cell ······ 232
gerontological ······244
gist191
glioblastoma ······220
global health ······203
glomerular density 55
glucose transporters ······ 60
glutamic acid ······12
goal directed therapy 143
gonadotropin-inhibitory hormone79
good clinical practice201
gout
gram-negative bacilli ······ 163
granule cells ······25
gravitational physioogy ······175
ground glass opacity
gut microbe ····································

H

hair
han 1/5
nand deteomity ······ 116
hand surgery ······116
HANS
HbA1c variability
HBV receptor ······151
head and neck reconstruction116
health guidance ······228
health literacy ······43
health working environment241
heart failure
heavy metal ······232
Helicobacter pylori ······155
helminth ·······40
hematopoietic stem cell ······22
hematopoietic stem cell transplantation 171
hemodialysis ······182
hepatocellular carcinoma
HER2
herpes simplex ······83

herpes zoster ······83
heterogeneous nuclear ribonucleoprotein ····· 210
high molecular weight hyaluronan ······ 179
high-pressure freeze fixation232
high-resolution manometry93
high-risk patients98
high-tech navigation operating room222
high-throughput screening
histone deacetylases ······69
HIV testing ······ 163
HLRW
home care nursing250
home medical care ······204
home visiting nurses249
hot spring ······218
human cytomegalovirus ······34
human herpesvirus 6 ······34, 79
human herpesvirus 7 ······34
human papilloma virus ······ 83, 139
hyperuricemia ······228
hypopharyngectomy ······116

I

I-BET151
idiopathic pulmonary fibrosis
iduronic acid ······187
IgA nephropathy
IL-31 receptor A
IL-6/IL-6R124
image-guided surgery system ······222
imaging ······37
immobile behavior ······232
immune checkpoint inhibitor191
immune response ······196
immunity ······40
infant cleaning care ······247
infection control ······249
inflammatory bowel disease48
innate immunity
institutional research ······2
insulin secretion ······224
integrated community care system73
interleukin-31 ····· 194
intermediate host ······40
international space station175
interstital lung diseases
interstitial cystitis ······129
intracranial stent device
iPS 187

ischemic heart disease
ischemic stroke ······196
Iternational Classification of Primary Care
Second Edition ······73

J

Japanese o	cedar pollinosis ······194
Japanese	language236

K

ketogenic diet ·····	25
kinase ·····	18

L

laminoplasty using novel plate spacers	105
laparoscopic colorectal surgery	93
laryngo microsurgery ······	139
laser ·····	83
latency ·····	
latency-associated transcripts	
lateral line neuromast ······	9
lattice vibration	233
life cycle ·····	216
lifestyle habits	228
liposome ·····	194
liver cirrhosis ······	
liver fibrosis	
liver transplantation	93
local circuit ······	••••••4
lung cancer ·····	69
lung cancer surgery	98
lymphadenopathy	73
lysosomal storage disease	187
Lysosome disease ·····	177

М

magnetic resonance imaging 199
magnifying endoscopic observation using a
narrow-band imaging155
malaria
malignant skin tumors ······83
marsupial ······167
mass spectrometry
maternal transport ······247
matrix
Matrix coil ······111
maxillofacial form167
MCT8 deficiency ······79
Mead, Gorge Herbert

mechanical valve ·····	119
medical and biological research	···· 218
medical communication	204
medical education ·····	2
medical English ······	238
medical image analysis	222
medical regulation	2
medulla ·····	•••••4
melanoma	194
menstruation	····· 247
mental disorder	
mental health ·····	244, 249
mental health and welfare act	···· 244
mentally ill patients	····· 249
metabolic ·····	····· 64
metabolomic analysis	187
metachromatic leukodystrophy	···· 206
metal photonic crystal ······	233
methicillin-resistant Staphylococcus aureu	s ···· 163
MIBG scintigraphy	
microarrays	
microcircuit ·····	•••••4
microdialysis ·····	75
microRNA-21 ·····	124
midgut ·····	
mineralization	9
mitochondria ·····	143
mitosis ·····	18
mitral valve plasty	119
molecular chaperone	
monitoring	230
monocarboxylate transporter	····· 199
Monte Carlo simulation	233
Morita therapy	75
mosquito ·····	
mouse embryonic fibroblasts	22
MRI ·····	196, 206
MRI contrast agent ·····	196
multiple system atrophy	
muscle ·····	175
musculoskeleltal system ·····	173
mutated tumor antigen	191
myosin ·····	12

N

nafamostat mesylate	191
narrow band imaging	139
nasal valve ·····	116
NASH·····	151

natriuretic peptide
nerve growth factor
neural crest cell ······9
neural network ·······4
neural stem cell
neurofibromatosis ······83
neuromuscular blocking agents143
neuron
neuropathic pain143, 199, 226
new anti hormonal agents
new drug
NF-κB ······187
NFκB inhibitor for hepatobiliary and
pancreatic surgery93
ningen dock ·······228
nitrogen cycle ······ 37
NMDA······4
nocardia ······177
non-alchoholic fatty liver
nonalcoholic steatohepatitis105
N-protected peptide acids234
nursing ······242
nursing administorater241
nursing administoration ······241
nursing diagnosis242
nursing education ······2
nursing ethics education245
nursing practice ······240
nutrition state ······73

0

obesity
objective structured clinical examination2
obstructive sleep apnea syndrome75, 139
occlusal discomfort ······167
occupational health nurse249
OLETF rat 173
open septorhinoplasty ······116
optic neuritis ······ 131
optic radiation131
optogenetics ······ 199
oral immunotherapy ·····194
organic chemistry234
outpatient nursing ······241, 245
outreach
ovarian clear cell adenocarcinoma 124
ovarian mucinous tumors
oxidative stress

p53 ······18 pain relief ·······179 parasite ······216 parliament ······235 patch-clamp ······25 pediatric palliative care79 peformance assessment ······2 perioperative nursing242 perioperative oral management167 personal identification46 phase transition12 phosphoglycerate kinase deficiency79 phosphorylation18 photon-phonon interaction233 physical assessment ······240 placode9 plasticity ·······226 polaprezinc ·······43 pollen tube cell ······232 polyamine ······22 polymerase chain reaction131 polymeric micelle ······196 polymyalgia rheumatica ······73 Polypterus ······9 porous random media233 positioning in nursing240 positron emission tomography75 postmastectomy pain ······143 postoperative pain ······226 postpartum ······247 prenatal genatic counseling124

pressure ulcer ····· 241, 244 primary aldosteronism ····· 55 primary biliary cirrhosis ····· 29, 48 primary endpoint ···· 230 primay-care ···· 204 programmed cell death ligand-1 ···· 191 PROM1 ···· 29

Propionibacterium acnes	
prosaposin deficiency	177
prostaglandin	15
prostate cancer	·· 29, 129
protection	
protein aggregate ·····	232
protocol	230
provide care ·····	
psoriasis ·····	
psoriatic arthropathy	
psychiatric nursing	244
psychotherapy	75
public health nurse	····· 249
pulmonary infarction	
pulmonary infection	
purinergic receptors	25

Q

QCM 12
quality assessment of medical care 204
quality index
quality management system
quality of life ······242

R

radiation-resistance
radioactive strontium ······218
radioisotope ······218
radiosensitization treatment
Radon
randomization ······230
randomized trial ······203
rat4
reactivation ····································
real-time imaging15, 222
red-flanked bluetail (Tarsiger cyanurus)233
regenerative medicine
renal failure ······ 182
renal transplantation55
renin-angiotensin-aldosterone system60
repetive transcranial magnetic stimulation 146
representations
research service providing system212
respiratory reflex ······25
revised guideline for traumatic brain injury ···· 149
rheumatoid arthritis
rhinoplasty ······ 116
Rho kinase ······64
rituximab ·······191

RNA aptamer ·····	·· 22
RNA binding protein	206
Roter Interaction Analysis System	236

S

sample size calculation230
sarcomere ·····15
sarcopenia ······173
sarcoplasmic reticulum15
scanning electron microscopy212
SCC measurement151
schizophrenia ······75
senescence ······69
sentinel lymph node
sentinel lymph node navigation
serotonin ······25
sexual intercourse resumption247
shared decision making236
simulation education ······2
single photon emission computed
tomography139
skeletal muscle ······12
skin care clinic83
skin gas224
sleep apnea syndromes ······167
small intestine
social resource
sonothrombolysis
space flight
spinabifida ······111
spinal cord injury206
spinal muscular atrophy210
sports injury in lower extremity179
sports-related concussion111
Staphylococcus aureus151
statin
statistical analysis230
statistical imaging analysis146
status epilepticus79
stem cells ······18
stereology ······4
stoma care ······241
stopping criteria
stroke
structural color ······233
subunit c of ATP synthase177
suffering ·······226
supine hypertension
supplement ······ 203

suprahyoid muscles ······167
surgeon's stress ······93
surgical simulator ······222
survival data analysis ······230
survival motor neuron ·····210
synapse ······4
synaptic plasticity199
synaptic transmission25, 199
syringomyelia ······111

Т

T cells
tapeworm ······40
Tardigrades ······218
targeting ······196
t-Branch stent graft 102
T-cell determinants
TDP-43
TeamSTEPPS ······163
tele-medicine software ·····111
telomerase ······ 58
temporomandibular disorders
Thioflavin T ······ 37
thrombin ······60
thrombolytic therapy
tick40
tissue valve ·······119
topiroxostat ······182
topological dynamics
topology optimization
total knee arthroplasty 105
tractus solitarius ·······
transcranial doppler
transcranial magnetic stimulation 226
transesophageal echocardiography 143
transforming growth factor
transfusion-associated circulatory overload ···· 171
transfusion-related acute lung injury171
transgenic rice
transmission electron microscopy212
transumbilical defunctioning ileostomy93
trastuzumab-emtansine ······191
traumatic brain injury149
Trojanow, Ilija ······239
twins with complete mole

ubiquitin
ubiquitin independent protein degradation ······22
ubiquitin proteasome ······173
ulcerative colitis48
ultra-high magnetic field MRI ······226
ultra-hypofractionation ····· 88
ureteral obstruction ······43
uric acid
urothelial carcinoma ······29
USP46
uveitis ······131

V

Vampirolepis nana
vector40
vestibular evoked myogenic potential139
video-assisted thoracic surgery
video-assisted thymectomy98
virus 40
visual field ······ 131
visual pigment ······131
vitamin B1
vitamin D 51, 203
Voice Handicap Index ·····139

W

water
WD-repeat protein ······232
weight control model for pregnant women ···· 247
Wilms' tumor 1 ······9
Winnicott, Donald Woods ······236
work environment ······240
work place ······240
WT1

Х

X-ray diffraction12

Z

zebrafish ·····	•9
Zeego ······1	11
Zinc ······	43
zinc finger ······ 1	87
Zucker fatty rat ······1	73