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Introduction

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

November 28, 2014

Continuing Medical Education Center
The Continuing Medical Education Committee

Hiroshi Tsuneoka, *Director*
Yashuo Toriumi
Rimei Nishimura
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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

1. Registered members: 222 (as of April 1, 2014)
   Members using the Center: 135/year
   Telephone service: 81 cases
2. The 34rd summer seminar was held on August 3, 2013. Eighty-one 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 (OSCEs), 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 Doctor Career Support is subdivided into the Office of Undergraduate Medical Education and the Office for Educational Development. The Branches contribute to undergraduate educational activities in the 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. To introduce systematic OSCEs to the Jikei University, the Center faculty researched year 3 and year 4 OSCEs used at the King's College London Medical School.
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 "Establishing 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.

3. Our project "Research on Nurse Practitioners Working with Cancer Specialists (physicians and pharmacists)" received a Supporting Grant for Clinical Cancer Research 2010 from the MHLW. We released research data on the quality of life of in-home patients serviced by nurse practitioners at the Japan Society for Medical Education conference in July.

4. Our proposal, "Building of General Practice Capability from Undergraduate to Life-long Learning: To Promote Clinical Research in the Community" was selected by MEXT to receive a Supporting Grant for New Paradigms: Establishing Centers for Fostering Medical Researchers of the Future Application 2013. The outline of the project is quoted from the proposal paper.

> "A program should be developed for training physicians with the capabilities of making/implementing clinical research plans to solve issues by themselves arising from community healthcare and publishing evidence based on expertise of general clinical care (i.e., "broad-ranging diversity") in a temporal axis from undergraduate to postgraduate/entire life by strong cooperation between the university and communities. This university has already introduced practical training, in which students can experience various medical needs in the community in a systemic manner early in their undergraduate education. Additionally, this university has conducted a clinical researcher training program for physicians who are engaged in community healthcare as continuing education. Therefore, this Project intends to develop and improve the school-wide system for training clinician researchers who are active in primary care settings and to establish centers for training human resources for community healthcare. These goals will be accomplished: 1) by expanding the "community healthcare experience training course" for undergraduate training and clinical training, 2) by creating a "general practice course" in the specialty training course (resident) in cooperation with a group of educational hospitals and facilities, 3) by establishing the coursework item "Primary care medicine for community healthcare" in the postgraduate physician's course, and 4) by creating a combined program for the graduate and specialty training course (resident)."

5. Workshop for team-building at a hospital: We organized workshops held in May (Nishi-shimbashi), June (Daisan), 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): Toho University School of Medicine (July), Showa University School of Medicine (August), Itabashi Medical System Group Patient Safety (August), Saitama Medical School (September), Kanazawa University School of Medicine (October), Hyogo Medical School (December), St. Marianna Medical School (January), Nara Medical School (January), Tokyo Medical and Dental University (February), Tohoku Bunka Gakuen University (March), and Nagasaki Nursing Center (March).

Department of Anatomy (Gross Anatomy and Neuroanatomy)

Yoshinori Kawai, *Professor* Toru 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 μm^2) 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 μm^2 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 μm^2; range, 62–120 μm^2; n = 22) and larger cells (mean somal area, 245 μm^2; range, 142–411 μm^2; 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 somatodendritic 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 arborization, as indicated by total dendritic length ($P < 0.01$) and the number of 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 encompass the small cell soma, whereas the number of axosomatic synapses decreases 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 local-

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.

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

Pathological and molecular biological investigation of congenital ataxia mouse bearing abnormal iron metabolism

Our congenital ataxia mouse had genetic alterations in the vicinity of rs13476689 in chromosome 2. Mutation analysis identified about 1,600 mutations, including single nucleotide polymorphisms, insertions, and deletions. In addition, a deletion of about 7,000 base pairs was found in the second intron of the Gm13912 gene. These genetic alterations made it possible to genotype individual animals to maintain the strain. However, the genetic alterations responsible for ataxia could not be determined. Vacuolar degenerations found in the spinal nerves and trigeminal nerves were the spheroid formation in the axons of NF200-positive neurons. The neurodegeneration had occurred before disease onset. Real-time polymerase chain reaction (PCR) analysis of gene expression suggested that iron deposition in the kidney was caused by decreased levels of divalent metal ion transporter.

The efficacy and the target specificity of genome editing with the CRISPR/Cas9 system

This year clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-mediated genome editing was developed for efficient genome editing in cells and organisms. We examined the efficacy and the target specificity of this method. We constructed CRISPR/Cas9 vectors targeting the human *HDAC8* and *NIPBL* genes, which are the responsible genes for Cornelia de Lange syndrome. We choose 2 highly specific target loci in each of *HDAC* and *NIPBL*. In addition, we predicted candidate off-target loci, including up to 3 mismatches against each target, using Bowtie, a short read mapping software program (available at http://bowtie-bio.sourceforge.net/index.shtml). We found a total of 64 potential off-target loci. We prepared multiplex primers for 4 target loci and 32 potential off-target loci for high-throughput deep sequencing.

We transfected the CRISPR/Cas9 vectors to HEK293 cells using FuGene HD transfection reagent (Promega Corp., Madison, WI, USA). Forty-eight hours after transfection, cells were harvested and the genomic DNAs were extracted. Then, multiplex PCR and

library construction were performed. High-throughput deep sequencing was performed with the Ion PGM system (Life Technologies, Carlsbad, CA, USA). We could obtain at least 10^4 reads with respect to each locus in each experiment. The efficacy of target-specific insertions/deletions ranged from 22.8% to 48.5%. On the other hand, off-target insertions/deletions were observed at a range of 0% to 3.1% (mean, 1.2%). Our results suggest that CRISPR/Cas9-mediated genome editing is highly active, even with imperfectly matched RNA-DNA interfaces in human cells. Therefore, when using CRISPR/Cas9 systems in research and therapeutic applications, careful evaluation of the off-target effects is required.

The vertebrate-specific structures—the neural crest and the placode—arise from the neural plate border: development of a new culture method for a possible precursor of exterior epithelium of the neural plate

Previous studies have shown that the neural crest is induced in the neural plate-embryonic ectoderm border by the action of bone morphogenetic protein (BMP) 4, which is derived from the embryonic ectoderm, and that the neural plate explant is likewise transformed to the neural crest cells by BMP4. We described a new culture method we developed and used it to find that the additive effects of BMP4 and fibroblast growth factor 2 on the neural plate explant results in morphological change to the simple squamous epithelium, which characteristically expresses Dlx5, which is a neural plate border specifier that positions the neural crest and future epidermis.

The induced epithelia were first tested by reverse transcription PCR (RT-PCR) for GATA3/keratin19 as epidermis specific markers, Sox1/Sox3/Neurogenin1/NCAM as neural plate markers, Slug/Snail1/Msx1/AP2/Zic1 as neural crest markers, and Dlx5/Six1/Six4/Eya2 as neural plate border markers using cells collected from neural plate explants and the corresponding control cells to evaluate the validity of RT-PCR testing. As a result, the expression levels of epidermis, the neural crest, and the neural plate border markers were all increased in the induced epithelia in telling contrast to those in the control cells.

We next examined the effect of Dlx5 downstream genes that are expressed in the neural plate and its border region on the induced epithelium by using real-time quantitative reverse transcription PCR (qPCR). The expression levels of epidermis specific markers GATA3/keratin19 and neural crest markers Slug/Msx1 in the induced epithelium were increased at the expense of the expression of neural plate marker Sox2. The pre-placodal ectoderm or ridge (PPE or PPR) arises in the anterior border of the neural plate in the form of an inverted-U shape and is regarded as a presumptive placode area at the late neurula-early pharyngula stage. The expression levels of the PPE-specific genes Six1/Eya2 known as the direct downstream genes of Dlx5, and of some placode-specific markers such as Pax3/Brn3a were also increased, albeit only slightly.

We lastly confirmed SEM images of the cultured cells, in which many epithelium-specific desmosomes were observed near boundaries of the adjoining big- and flat cells, which were cultured with BMP4 and FGF2 as the experimental group, although in which many filopodia and lamellipodia were surrounding cells, which were cultured with BMP4 as the positive control group to form motile neural crest cells.

This study thus suggests that the neural plate cells have a latent ability to be transformed into exterior epithelium of the neural plate, such as the neural crest, the PPE, and the embryonic ectoderm through the action of BMP4 and FGF2. The induced epithelium might be a precursor of all exterior epithelium of the neural plate. We are now investigating molecular cascades and cell differentiation in both the epithelium induced by the explant culture and the neural plate border in the embryo.

Analysis of cell distribution in diaphragm development

The diaphragm consists of mesodermal tissues from several sources. Because of its complexity, the development of the diaphragm remains unclear. For this reason, we observed the distribution of cells that are involved in diaphragm development. We used the Wilms' tumor 1 (*Wt1*) gene, because it is the responsible gene for congenital diaphragmatic hernia. We observed *Wt1*-positive cells in the left side of posterolateral region, which is the most common site of congenital diaphragmatic hernia. We plan to analyze the mechanism of the distribution of *Wt1*-positive cells in this region.

Formation of vertebrate appendages (limbs and fins) during development and regeneration

Limbs and fins, which are paired appendages of gnathostomes used for locomotion, are formed by shared developmental mechanisms, although the final morphologies of limbs and fins differ from each other. While limb bones are formed by endochondral ossification, fins comprise both endochondral bones and intramembranous bones. To understand the mechanisms of fin bone development and fin-to-limb evolution in gnathostomes, we are investigating pectoral fin formation in zebrafish (*Danio rerio*). We found a mutant fish whose pectoral fin lacked intramembranous bones.

In adults, limbs (of amphibians) and fins can regenerate completely by about 2 weeks after amputation. Previous work indicates that regenerative fin growth is greater after proximal amputation than after distal amputation. Therefore, we investigated levels of gene expression during such position-dependent regeneration by using quantitative polymerase chain reaction analyses. We found that the position-dependent regeneration already occurred in the inflammatory period before the blastema proliferation period. We would like to find a candidate for factor during position-dependent regeneration and to understand similarities and differences of morphogenesis during development and regeneration.

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Reviews and Books

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Department of Molecular Physiology

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

To reveal the properties of interaction in the water component of skeletal muscle sarcomeres distinguished by ^{1}H-nuclear magnetic resonance measurement, we observed the phase transition of water in skeletal muscle by means of differential scanning calorimetry, which provides direct information about the interaction energy between molecules. We found significant enthalpy change at around the melting point of water (0˚C) and at lower temperatures (− 25˚C). The enthalpy change at − 25˚C on fresh specimens was smaller than that on denatured specimens. Furthermore, the difference in enthalpy between fresh and denatured specimens almost coincided with the denaturing enthalpy at > 40˚C. The fresh specimens would have a specific heat capacity 0.04 J/˚C/g higher than that of the denatured specimens. This large excess heat capacity would reside in myoproteins and in water molecules, which bear large entropy, and might serve as a heat sink to support muscle contraction.

Viscoelastic properties of water around myosin

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). When myosin was adsorbed more sparsely than 0.2 μg/cm^2, the viscoelastic change accompanying the myosin adsorption was almost the same as the viscoelasticity of buffer without myosin. The resonance frequency fell to the level equivalent to weight of the adsorbed myosin. 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, a large viscoelastic change was observed. Viscoelastic analysis indicated that myosin acts as a protein having viscoelasticity and that the binding of ATP to myosin heads changes the viscoelasticity of the protein. This finding suggests that myosin immobilizes the surrounding solution when adsorbed closely. Half of this immobilized solution was released in the presence of ATP or ADP but not in the presence of ATP-γS.

Structural change of thick filaments in thin-filament-extracted skinned fibers during the ATP hydrolysis cycle

To clarify the mechanism by which myosin heads convert the chemical energy of ATP to mechanical work, we performed an X-ray diffraction study of myosin heads in skeletal muscle fibers without actin filaments to examine structural changes in myosin during the ATP hydrolysis cycle without interaction with actin. When ATP bound to myosin heads, the peak of the myosin layer line shifted outward, indicating the shift of the center of gravity of myosin heads toward their rods accompanying the transition from the state of myosin without nucleotide ("M") to that of myosin with ADP and Pi ("M-ADP-Pi"). The binding of ADP to myosin heads did not shift the peak of the myosin first layer line, indicating that the myosin structure did not differ significantly between the state without nucleotide ("M") and that with ADP ("M-ADP"). Treatment with N-phenylmaleimide, which shifts myosin heads to the ATP-bound state ("M-ATP") from the ADP-Pi-bound state ("M-ADP-Pi"), slightly shifted the peak of the myosin first layer line to resemble that in the "M" state, indicating that the binding of ATP to nucleotide-free myosin itself does not change the structure of the myosin heads but that the succeeding hydrolysis step of ATP does induce structural change of the myosin heads.

Role of polyamines in skeletal muscle cell proliferation and differentiation

The polyamines putrescine, spermidine, and spermine are low molecular weight organic polycations and mediators of cell homeostasis. The polyamines have been proposed to play roles in the functioning of ion channels, nucleic acid packaging, signal transduction, autophagy, DNA and protein synthesis, cell proliferation and differentiation, and the regulation of gene expression. Although the regulation of polyamine levels is associated with skeletal muscle hypertrophy, the underlying mechanisms of polyamines have not been well defined. Here, we studied how polyamines affect the proliferation or differentiation or both of the murine myoblast progenitor C2C12 cell line. To evaluate the role of polyamines in the proliferation process, we counted the number of myoblasts every 24 hours, but polyamines had no effect on myoblast proliferation. On the other hand, during the induction of myogenic differentiation, treatment of C2C12 cells with polyamines significantly increased the number of myotubes. Polyamine-treated C2C12 cells exhibited elongated cell bodies and became multinucleated myotubes. Ultrastructural analysis with transmission electron microscopy revealed that polyamine-treated multinucleated myotubes exhibited abundant myofilaments. Therefore, our study suggests that polyamines play an important role in regulating myogenic differentiation rather than myoblast proliferation.

Publications

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Reviews and Books

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Department of Cell Physiology

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

General Summary

The aim of research in our laboratory is to understand the regulatory mechanism of the cardiovascular system. In particular, we are interested in the development of the cardiovascular system, the mechanics of sarcomere contraction, Ca^{2+} homeostasis in the cardiac sarcoplasmic reticulum, and the pathophysiology of cardiac fibrosis. We established an experimental system to investigate small fetal arteries, such as the rat fetal ductus arteriosus (DA). 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 (PGE_2) 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. In addition, using a fluorescence-activated cell sorter, we isolated endothelial cells from pooled tissues from the DA of fetal Wistar rats. We found several significant differences in transcriptional profiles between the DA and aortic endothelial cells. Newly identified DA endothelium-dominant genes 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 found that selective blocking of PGE_2, in particular, EP4 prostanoid receptor signaling, attenuated the development of AAA. We are examining the molecular mechanisms using animal models, such as the calcium chloride-induced AAA rat model and Brown-Norway rats that exhibit irregular internal elastic laminae.

Regulation of cardiac sarcoplasmic reticulum ATPase activity

Impaired Ca^{2+} reuptake into the sarcoplasmic reticulum is thought to be a primary pathogenic mechanism of heart failure. We are interested in sarcolipin, a regulator of the sarcoplasmic reticulum Ca^{2+}-ATPase, that is specifically expressed in atrial muscle. We generated a knockout mouse to insert a gene-targeting vector containing the cassette of the Cre recombinase gene into an endogeneous sarcolipin locus by homologous recombination. This mouse enables us to generate atrium-specific gene targeting. We are characterizing the phenotypes of sarcolipin knockout mice.

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. Furthermore, the atrium and ventricle have chamber-specific functions, structures, gene expressions, and pathologies. The left ventricle works as a high-pressure chamber to pump blood toward the body, and its muscle wall is thicker than those of the other chambers, suggesting that energy utilization in each chamber should be different. Using capillary electrophoresis and mass spectrometry, we found that overall metabolic profiles, including nucleotides and amino acids, were similar between right and left ventricles. On the other hand, the atria exhibited a metabolic pattern distinct from those of the ventricles. Importantly, the high-energy phosphate pool (the total concentration of ATP, ADP, and AMP) was higher in both ventricles. Accordingly, the activities or expression levels or both of key enzymes were higher in the ventricles to produce more energy. Our findings provide a basis for understanding the chamber-specific metabolism underlying pathophysiology in the heart.

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. The peak Ca^{2+} in both the interstitial fibrosis and nonfibrosis groups was significantly higher than that in the control group. However, peak tension in the interstitial fibrosis group was significantly less than that in the nonfibrosis and control groups. The time to peak Ca^{2+} in the interstitial fibrosis group was significantly longer than that in the nonfibrosis and control groups. Immunohistochemical staining showed that connexin 43 accumulation in the intercalated disks was less in the interstitial fibrosis group than in the nonfibrosis and control groups. These results indicate that impairment of tension development of cardiac muscle with interstitial fibrosis is due to lower Ca^{2+} sensitivity and less cell-to-cell communication.

Mechanism of sarcomere contraction in cardiac muscle

1. Depressed Frank-Starling mechanism in left ventricular muscle of the knock-in mouse model of dilated cardiomyopathy with troponin T deletion mutation ΔK210

We investigated how the sarcomere length-dependence of active force production is altered in a knock-in mouse model of inherited dilated cardiomyopathy (DCM) with deletion mutation ΔK210 in the cardiac troponin T gene. Confocal imaging revealed that the cardiomyocytes were significantly enlarged, especially in the longitudinal direction, in the hearts of ΔK210 knock-in mice, with striation patterns similar to those in wild-type hearts, suggesting that the number of sarcomeres is increased but their length remains unaltered. To analyze the sarcomere length-dependence of active force, skinned muscles were prepared from the left ventricles of wild-type and ΔK210 mice. Accordingly, we found that the depressed Frank-Starling mechanism in the hearts of ΔK210 knock-in mice is the result of reduced thin-filament cooperative activation.

2. 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 cardiomyocytes under various settings.

3. *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

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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 these myriad signals. Normal proliferative cells are endowed with the ability to choose from among growth, 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 from among proliferation, differentiation, and apoptosis.

Research Activities

Induction of amphiregulin by p53 promotes apoptosis via control of microRNA biogenesis in response to DNA damage

Tumor suppressor p53 functions as a transcription factor to induce its target genes modulating cell-cycle arrest, DNA repair, and apoptosis induction. Selective transactivation of p53 target genes is determined by posttranslational modifications of p53. In particular, Ser46 phosphorylation is a requisite for commitment to induce apoptotic cell death. To clarify the mechanism of p53-mediated apoptosis, we explored target genes that are induced in a Ser46 phosphorylation-specific manner. By means of chromatin immunoprecipitation analysis, we demonstrated that amphiregulin (*AREG*) is a novel target gene for p53. To investigate the mechanism of AREG-mediated apoptosis induction, we analyzed AREG-interacting proteins by mass spectrometry. One candidate, DEAD box protein 5 (DDX5), was found to co-localize with AREG in the nucleus. DDX5 plays an essential role in precursor microRNA processing. Intriguingly, AREG regulates microRNA biogenesis (i.e., miR-15a) in response to DNA damage.

Dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 controls the epithelial-mesenchymal transition and metastases in breast cancer by degrading Snail

The epithelial-mesenchymal transition (EMT) plays a fundamental role in the early stages of breast cancer invasion. Snail, a zinc finger transcriptional repressor, is an important regulator of EMT. Snail is phosphorylated by glycogen synthase kinase 3β and is subsequently degraded by β-transducing-repeat-containing protein-mediated ubiquitination. We identified an additional kinase, dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 (DYRK2), which regulates Snail stability. We stably silenced DYRK2 in MCF-7 cells (short hairpin RNA-DYRK2 cells). Stable DYRK2 depletion led to Snail accumulation and decreased E-cadherin in MCF-7 cells. Knockdown of

DYRK2 promoted the EMT and cancer invasion in vitro. In MDA-MB-231 cells, the overexpression of DYRK2 reduced the invasive capacity. Our results suggest that DYRK2 phosphorylates Snail at Ser104 as a priming phosphorylation for glycogen synthase kinase 3β. In a xenograft model, a significant increase in bone and lung metastasis was observed in the DYRK2-shRNA group. Consistent with these results, DYRK2 was found to be down-regulated in human breast cancer tissue. Patients with low DYRK2-expressing tumors had a worse outcome than did patients with high DYRK2-expressing tumors. These findings suggest that DYRK2 regulates cancer invasion and metastasis by degrading Snail.

E-cadherin suppression in epoxomicin-resistant cells may be regulated by expression of ZEB1

Our previous study demonstrated that in endometrial carcinoma Ishikawa cells resistant to the proteasome inhibitor epoxomicin (Ishikawa/EXM cells), E-cadherin was suppressed via expression of the transcriptional repressor gene *ZEB1*. Down-regulation of E-cadherin plays an important role in the EMT. The expression of zinc finger E-box-binding homeobox (ZEB) 1 was concerned with the suppression of dual-specificity protein phophatase 6 (DUSP6) via extracellular signal regulated kinase (ERK) 1/2 signal transduction. Because we found DUSP6/MAP kinase phosphatase (MKP) 3 disappearing in Ishikawa/EXM cells, we studied the participation of DUSP6/MKP3 in E-cadherin expression in Ishikawa/EXM cells. Suppression of DUSP6 and expression of FOS-like antigen 1 (Fra1) were observed in Ishikawa/EXM cells. It was reported that activated ERK2 was upregulated ZEB1/2 following phosphorylation of Fra1. Both knock-down of DUSP6 by treatment of Ishikawa cells with short interfering RNA for DUSP6 and inhibition of DUSP6 activity by treatment of Ishikawa cells with (E)-2-benzylidene-3-(cyclohexylamino)-2,3-dihydro-1H-inden-1-one (BCI), an inhibitor of DUSP6 activity, induced expression of Fra1 by activation of ERK2 and induced repression of E-cadherin following expression of ZEB1. Moreover, overexpression of Fra1 in Ishikawa cells transfected with Fra1/pcDNA3.1 caused ZEB1-induced suppression of E-cadherin. On the other hand, expression of DUSP6 by transfection of Ishikawa/EXM cells with DUSP6/pcDNA3.1 induced expression of E-cadherin following suppression of Fra1 and ZEB1. These results suggest that the disappearance of DUSP6 in Ishikawa/EXM cells causes up-regulation of ZEB1 via the expression of Fra1 and induces the EMT.

Development of an efficient production system of fibrinogen using a hepatocellular carcinoma cell line

With the increasing demand for blood products globally, transfusions are not without risk because blood products that are mostly dependent on blood donations can be associated with infectious disease transmission. Therefore, the development of an effective production system to ensure the safe and plentiful supply of blood products is important. We attempted to develop an effective fibrinogen production system that satisfied these requirements using human functional hepatic cell lines (FLC-7) and serum-free medium (ASF104N and IS-RPMI). Massive fibrinogen production was induced with a

radial flow bioreactor. In the radial flow bioreactor culture, the fibrinogen secretion rate reached 109.5 μg per day during a 42-day cultivation period. The subunit composition and clot formation activity of FLC-7 cell-derived fibrinogen corresponded to those of plasma-derived fibrinogen. Thus, the system we developed is suitable for large-scale fibrinogen production.

Study of deubiquitinating enzyme ubiquitin-specific protease 46 underlying despair behavior in mice

Ubiquitin-specific proteases (USPs) are deubiquitinating enzymes that remove ubiquitin from specific protein substrates and modulate the ubiquitin-proteasome system. Recently, *Usp46* was identified as a quantitative trait gene responsible for decreasing tail suspension test and forced swimming test immobility time in CS mice. The CS mouse has a 3-bp deletion coding for Lys 92 of the protein USP46, but the effect of the deletion mutation on deubiquitinating enzyme function is unclear. To investigate the structural basis of the deletion of Lys 92 in USP46, we used the homology-modelling program SWISS-MODEL and predicted the 3-dimensional structure of USP46 based on the crystal structure of USP21. In the obtained model, Lys 92 was located at the loop structure on the surface of USP46 and was distant from the active site. This model suggests that Lys 92 mediates the interaction of USP46 with the target or partner proteins. To identify the proteins interacting with USP46, we first generated a stable neuroblastoma cell line (SH-SY5Y) expressing Flag-USP46 (wild-type and mutant). Then, we screened the interacting proteins using liquid chromatography/tandem mass spectrometry analysis, combined with the co-immunoprecipitation method. Our analysis identified several endogenous proteins associated with USP46. For example, WD repeat domain 48 and dystrophia myotonica, WD repeat-containing, interacted with either wild-type or mutant Flag-USP46. Detailed studies are currently in progress.

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Department of Molecular Biology

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General Summary

Polyamines (putrescine, spermidine, and spermine) are ubiquitous biogenic amines that bind mainly to nucleic acids and are essential for cell proliferation. Cellular polyamine contents are maintained by a feedback mechanism involving the key regulatory proteins antizymes (AZs). The AZs are expressed by translational frameshifting that is induced by polyamines and negatively regulate cellular polyamines. Three AZ isoforms (AZ1-3) are present in mammals. 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 ubiquitin-independent manner. To clarify the significance of this interaction in the nucleus and nucleolus, we investigated the effects of knockdown of AZ2 or c-MYC on the subcellular localization of each protein. Interestingly, localization of AZ2 was shifted from the nucleus or nucleolus to the cytoplasm by c-MYC knockdown. We further investigated whether AZ2 accelerates c-MYC degradation in the nucleolus. Nucleophosmin 1, which is important for maintaining the nucleolus localization of c-MYC, was overexpressed in the cells, and then c-MYC degradation was monitored with overexpression or downregulation of AZ2. Overexpression of AZ2 increased degradation of endogenous c-MYC, whereas downregulation of AZ2 suppressed it. These results indicate the possibility that AZ2 accelerates the degradation of c-MYC in the nucleolus.

Analysis of interaction between AZ2 and ATP citrate lyase
ATP citrate lyase (ACLY) generates acetyl-CoA from mitochondria-derived citrate and is important for fatty acid synthesis and histone acylation. We have identified ACLY as an AZ2-interacting protein. To confirm a direct interaction of these proteins, we performed an *in vitro* pull-down assay using purified hemagglutinin-tagged ACLY, AZ1, and AZ2. Interestingly, ACLY bound not only to AZ2 but also to AZ1. To investigate how AZs affect ACLY activity, we measured ACLY activity *in vitro* and *in vivo* in the presence or absence of AZs. In the presence of AZs, ACLY activity was nearly doubled, and knockdown of AZs caused a decrease in ACLY activity. Polyamines did not affect

ACLY activity *in vitro*. These results suggest that AZs directly bind to ACLY and regulate ACLY activity.
We are also developing a method for measuring ACLY activity with a mass spectrometer using stable isotope-labeled citrates as a substrate.

Detection of posttranslational modification by polyamine
Polyamines covalently bind to proteins at glutamine residues by a transglutaminase reaction. This posttranslational modification by polyamine, namely polyamination, may change the activity or property of the protein. Little is known about target proteins and the physiological significance of the polyamination. We developed methods to detect polyamination. With putrescine-bound dimethyl-casein prepared by transglutaminase reaction as a sample, the molecular-related ion of gamma-glutamylputrescine was accurately detected with liquid chromatography mass spectrometry and a multimode octadecylsilyl column. Furthermore, the increased molecular mass by polyaminations at the 2 glutamine residues in a peptide fragment was detected by liquid chromatography tandem mass spectrometry analysis with low-flow captive spray ionization.
Mass-based global analyses of polyamination during changes in cellular polyamines and quantitative proteomics using stable isotope labeling will provide clues to the physiological significance of polyamination.

Multiple forms of mouse Azin1 messenger RNA differentially regulated by polyamines
We have found multiple forms of *Azin1* transcripts formed by alternative splicing and initiation of transcription from putative alternative start sites. This year, we first showed that stability of *Azin1* messenger (m) RNA was largely consistent in the presence or absence of 2-difluoromethylornithine, an inhibitor of polyamine synthesis. This finding indicates that polyamine does not affect the stability of *Azin1* mRNA. Next, to evaluate the physiological importance of Azin1, we examined the cell growth, in wild-type mouse embryonic fibroblasts ($MEFs^{+/+}$), and mutant MEFs ($MEFs^{-/-}$) in which expression of Azin1 is largely diminished. We found that cell growth rate of the mutant MEFs was increased by polyamine and by thymidine. We are now comparing expressed protein and metabolite profiling in both MEFs.

Isolation and analyses of polyamine-binding RNA aptamers
RNA aptamers have the potential for both clinical and research applications. In particular, 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 proposed a model of binding between spermine and a previously isolated anti-spermine aptamer. In this model 2 separated stem regions form 1 spermine-binding site with spermine-induced conformational change putting the 2 stems closer. This year, we performed stoichiometric analyses using isothermal titration calorimetry or a quartz crystal microbalance. From isothermal titration calorimetry, the specific 1:1 interaction between the aptamer and spermine was detected. With this assay, the dissociation constant (*K*d) was estimated to be 250 μM. With the quartz crystal microbalance assay, the specific inter-

action was also detected. However, the detected interaction value was much greater than expected. This greater value may come from some conformational change of the RNA aptamer induced by its interaction with spermine. These results support the binding model of this aptamer and suggest that spermine can induce conformational changes of RNA molecules that are the major binding partner for polyamines.

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Department of Pharmacology

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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 glucocorticoid production by activation of purinergic receptors in a human-derived adrenocortical cell line (Haruhisa Nishi)
5. Functional significance of stripe zones of the cerebellar cortex (Taro Ishikawa)
6. The basic mechanism underlying the anticonvulsant effects of a ketogenic diet (Masahito Kawamura)
7. The effect of cerebrocerebellar connections in the cerebellar input system (Misa Shimuta)

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 fish with lungs, unlike mammals, never open their glottis during yawning. Sharks, which lack lungs, also show yawning-like behavior. "Yawning" in 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 a more primitive jawless fish, the lamprey. Lampreys, when they stopped sucking the wall of a 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 an endogenous anxiogenic peptide that could suppress GABA binding to $GABA_A$ receptors 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 messenger RNA from bovine adrenocortical cells, and the complementary DNA of endozepine was amplified with the polymerase chain reaction for insertion into 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. We would like to examine the posttranslational modifications and intracellular functions of endozepine expressed by mammalian cells.

Study of glucocorticoid production by activation of purinergic receptors in a human-derived adrenocortical cell line

The human adrenocortical cell line NCI-H295R was used to study the function of purinergic systems in human adrenocortical steroidgenesis. We found that H295R cells express a functional P2Y1 purinergic receptor for intracellular Ca^{2+} mobilization and that $P2Y_1$ is linked to store-operated calcium entry activation, leading to Ca^{2+} influx. The latter might be a key for glucocorticoid secretion. These findings indicate that functional purinergic systems and the crosstalk of intracellular second messengers for steroidogenesis exist in human adrenal cortex cells. This study was previously reported in PLoS ONE (PLoS ONE (2013), 8(8): e71022.). Both the efficiency of the $P2Y_1$ knock-down system and the effects of ectonucleotidase activity in H295R were reevaluated and were reported at the 87th annual meeting of the Japanese Pharmacological Society (Sendai, March 20, 2014).

Functional significance of stripe zones of the cerebellar cortex

The mammalian cerebellar cortex has sagittal zones (vertical stripes) that are recognized by the expression of the glycolytic enzyme aldolase C. Recent studies have revealed

that these zones differ in their neural connections to the cerebellar nuclei and the brainstem. However, the functional significance of such differences is not clear. To investigate this issue, we are conducting experiments (in collaboration with Tokyo Medical and Dental University) in transgenic mice whose zonal structures are visualized through the expression of fluorescent protein, and using electrophysiological techniques, including in vivo patch-clamp recording.

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.

The effect of cerebrocerebellar connections in the cerebellar input system

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. Therefore, we investigated the relationship between the cerebral activity and the cerebellar inputs by simultaneously recording in vivo the field potential of the cerebral cortex and the whole-cell synaptic currents of cerebellar granule cells. We found that the cerebral activity and the cerebellar synaptic inputs have periodical spontaneous activities. Our findings suggest that single granule cells can integrate the signals from the periphery and the descending signals from the cerebral cortex. On the basis of these findings, we are conducting research to clarify the significance of the cerebrocerebellar connections.

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General Summary

The research objectives inthe Department of Pathology include the investigation of pathogenesis and morphological changes based on morphology. The materials used include human specimens from autopsies, surgical resections, and biopsies. These materials are examined with light microscopy, electron microscopy, morphometry, immunohistochemistry, and molecular pathology.

Research Activities

Research in liver disease

1. In the evaluation of primary biliary cirrhosis (PBC), Scheuer's classification and Ludwig's classification have traditionally been used, but Nakanuma's classification has now recently been proposed. An increase in serum anti-gp210 antibodies in patients with PBC is associated with more frequent progression to liver failure and lower survival rates. In the present research, we have evaluated PBC activity and staging using Nakanuma's classification and compared this with Scheuer's classification. We have also examined histological findings in high and low anti-gp210 antibody groups to investigate how anti-gp210 antibody levels are related to histological findings. In Nakanuma's classification, various histological findings in PBC are scored and totaled to comprehensively assess activity and staging. The problem with this classification is that it requires complex evaluation. In the high anti-gp210 antibody group, the rate of chronic nonsuppurative destructive cholangitis, the fibrosis score, and stage were all higher. This finding confirm a strong correlation between these antibodies and the progression to liver failure.
2. Serial sections were prepared from biopsy specimen blocksfrom patients with early chronic hepatitis. Through histologic reconstruction of these serial sections, we examined the type of injury that occurs in portal vein branches in chronic hepatitis. We found that in early chronic hepatitis, these portal vein branches are lost within areas of fibrosis.
3. Using image analysis, we measured arterial density, arterial wall thickness, and the inner/outer diameters of arterial lumens in normal livers to investigate their relationships with aging. However, we found no obvious association with age. We also examined the number of nuclei in hepatocytes in fixed areas of the hepatic parenchyma and found that hepatocyte size increased with aging.
4. With a focus on protein metabolism in nonalcoholic steatohepatitis, we examined

metabolic impairment in needle biopsy liver specimens from the perspective of oxidative stress and glycation stress. Dityrosine, a protein oxidation marker, was uniformly distributed as granules in hepatocytes. These granules were densely distributedaround areas of fibrosis and in hepatocytes near the portal tract. Carboxymethyl lysine, a glycoxidation marker, showed a similar distribution and was even more densely distributed in phagocytic macrophages.

Research in kidney disease

1. The Oxford International Classification for immunoglobulin (Ig) A nephropathy differs from the histological severity classification in Japan (Japanese Classification). We performed a retrospective study of cases in Japan using the Oxford International Classification. This study included 233 adult patients with IgA nephropathy over a follow‐up period of 127 months. With a 50% decline in renal function or progression to end‐stage renal disease as an endpoint, Cox simple regression and multiple regression analyses were performed. We found that extracapillary lesions and the severity of interstitial fibrosis were independent indicators of renal prognosis. However, in the Oxford Classification, interstitial fibrosis, segmental sclerosis, mesangial hypercellularity, and endocapillary proliferation, rather than extracapillary lesions, were indicators of a poor prognosis. The difference between classifications is attributed to differences in follow‐up period, patient age, initial estimated glomerular filtration rate, and restrictions in protein.

Research in gastrointestinal disease

1. For neuroendocrine neoplasms of the colon, the World Health Organization gastrointestinal tumor classification (2010) recommends diagnosis based on a combination of mitotic number and Ki‐67 index of the lesions; these neoplasms are classified as neuroendocrine tumorsgrade 1 and grade 2 and neuroendocrine cancer. Therefore, we reviewed lesions at our hospital previously diagnosed as carcinoid tumors, applied the new diagnostic criteria, and examined the pathological features. The lesions included 98 colon carcinoid tumors that had been endoscopically resected. We found that higher‐grade neuroendocrine tumors were larger and had higher rates of lymphatic and venous permeations.
2. We investigated risk factors for lymph node metastasis of small colon cancers in 203 patients with submucosal (SM) cancer and 62 patients with more advanced carcinoma measuring ≤ 20 mm. Six risk factors for lymph node metastasis in SM cancer were established: macroscopic depression, intramucosal growth (polypoid growthor nonpolypoid growth type), depth of SM invasion (SM1 or SM2/3), histological invasion type (well/moderate or poor), budding, and lymphatic and venous permeations assessed using immunostaining. These factors were subjected to multivariate analysis. The most important risk factor was lymphatic and venous permeations (odds ratio: 9.5). In almost all cases without lymphatic and venous permeations, lymph node metastasis was absent. The findings were similar for advanced carcinoma ≤ 20 mm. The results suggest that for lesions without lymphatic and venous permeations, the risk of lymph node metastasis islow.

Research in genitourinary disease

1. Immunohistologic studies were performed in 8 patients with inflammatory myofibroblastic tumors of the bladder. Immunostaining for anaplastic lymphoma kinase was positive in all patients, and in 1 of these patients, p53 was diffusively positive and mitoses were prominent. This patient had multiple recurrences during follow-up, and histopathologic examination showed changes with a high grade of atypia. Therefore, staining for p53 may be useful as a marker for malignancy in inflammatory myofibroblastic tumors.
2. In 201 Japanese men with prostate cancer, we examined clinicopathologic factors according to cancer location. Eighty-three patients had anterior cancer, 72 had posterior cancer, and 46 had other types of cancer. Compared with Western countries, Japan has a higher prevalence of anterior prostate cancerThe distribution of Gleason scores and pT stages differed significantly between anterior and posterior cancers. However, even in anterior cancer, in a few patient of there showed high Gleason scores and pT stages at rates cannot be ignored.
3. From 1,344 patients with placental lesions evaluated at our hospital, we extracted data from 21 cases of placental hemangiomas to investigate clinicopathologic features. Placental hemangiomas were more common in women who were primiparas, had multiple births, or had given birth to a girl; and hemangioma size was positively correlated with risks for fetal hydrops and right heart load. Histologic examination showed that most lesions were capillary hemangiomas. One case had features of a lymphangioma or lymphatic malformation.
4. We reviewed clinicopathologic features in 12 cases of malignant mesonephric tumors. Half of these tumors had arisen in the uterine cervix, but other primary sites included the uterine corpus, ovary, and paraovarian structures. Mesonephric remnants or hyperplasia was seen in only 4 patients. Histologic examination often showed a variegated appearance with features resembling endometrioid adenocarcinoma. Some tumors were composed mainly of spindle cells or resembled carcinosarcoma. The histogenesis included tumors derived from mesonephric remnants or hyperplasia, and adenocarcinoma with differentiation into a mesonephric tumor was considered.
5. We examined the histologic features of placental mesenchymal dysplasia at 21 weeks or less of pregnancy. Compared with pregnancy at week 20 and later, characteristic findings included changes resembling a partial hydatid mole and blood vessel proliferation in the villous stroma.
6. Luteinized thecomatosis with sclerosing peritonitis is a rare disease characterized by bilateral ovarian luteinized thecomas and peritonitis. Some authors suggest that these ovarian lesions may be either neoplastic or reactive, but no consensus exists. Whether the ovarian lesions are related to the peritoneal lesions is also unknown. Histologic examination in our case showed proliferation of spindle cells, mixed with some luteinized oval cells, in the ovarian cortex bilaterally. The oval cells had features of a thecoma, but uniform proliferation suggesting a tumor could not be confirmed; therefore, it was also difficult to distinguish between a neoplastic lesion versus a reactive lesion in our patient. In addition, the association between ovarian and omental lesions is unclear.

Other research

1. We performed an autopsy in a case of Chagas disease diagnosed while the patient was still alive. Amastigotes were found in the myocardium and brain. This patient had a history of long-term overseas business trips. Therefore, with the increase of persons born and coming from abroad or returning from abroad to Japan, prophylaxis against Chagas disease should be considered.
2. We encountered a case of angiomatoid fibrous histiocytoma in the left upper arm of a 7-year-old girl. Although this tumor is rare, it was diagnosed on the basis of characteristic clinical and pathological findings. Serum levels of interleukin 6 levels were elevated before surgery but decreased after surgery. Immunohistologic staining showed that the tumor was positive for interleukin.

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Department of Virology

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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 the pathogenesis of HHV-6 and searching for latent proteins 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

Novel HHV-6 stress-related latent protein induces depression and suicide

Background: HHV-6 is one of a few viruses that establish latency within the brain. Latent infection occurs in astrocytes, and chronic stress can cause virus reactivation. However, the effects of reactivation in individuals with normal immune function were previously unknown. We identified the HHV-6 latent transcript that was expressed during the relatively activated latent stage (intermediate stage) of HHV-6 latency. This transcript encoded a small open reading frame, which we named small protein encoded by the intermediate-stage transcript of HHV-6 (SITH) 1.

Objective: To clarify the function of SITH-1 in the brain.

Methods: We studied the expression of the SITH-1 by examining the prevalence of antibodies against it among suicide attempters and healthy individuals. Next, an open reading frame of SITH-1 was linked downstream of a glial fibrillary acidic protein promoter and expressed in glial cells of mice using an adenovirus vector. After growth, the behavior and gene expression of the mice were analyzed.

Results and Discussion: The positivity rate of the anti-SITH-1 antibody was high in suicide attempters, especially those in whom depression had been diagnosed. Mice expressing SITH-1 showed a decrease in spontaneous motor activity and an increase in immobility time in the tail suspension test. Moreover, SITH-1 mice showed reduced expression of brain-derived neurotrophic factor messenger (m) RNA. Therefore, astrocytes expressing SITH-1 seem to play a major role in depressive-like behavior in mice. These results suggest that SITH-1 is involved in the onset of mood disorders. Our findings suggest that SITH-1 expression itself induces depression and, therefore, is a risk factor for depression and suicide.

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

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 SITH-1, which is produced specifically in astro-

cytes 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 promoter-driven 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 mRNAs 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 corticotropin-releasing hormone mRNA and, interestingly, significantly decreased bcl-2 mRNA and increased apoptotic cells (as indicated by terminal deoxyribonucleotidyl transferase-mediated deoxyuridine triphosphate-fluorescein nick-end labeling) in the olfactory bulb. Overall, stress increases 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.

Human cytomegalovirus latency-associated protein ORF152 induces calcium signaling and promotes differentiation of myeloid progenitor cells
Human cytomegalovirus (HCMV) infection of healthy individuals is usually asymptomatic and results in the establishment of a life-long latent infection. Granulocyte-macrophage progenitors are sites of latent HCMV infection. The viral genome persists in these cells with highly restricted viral gene expression and no detectable virus production. Viral reactivation from latency is closely associated with cell differentiation. Sense and antisense CMV latency-associated transcripts, which are detected in latently infected cells, have been mapped to the ie1/ie2 region of the HCMV genome. One of the antisense transcripts, ORF152, is conserved among HCMV strains, but its role during latency remains unclear.
Here, we report the function of the HCMV ORF152 protein, which is expressed during latent infection. A yeast 2-hybrid screen showed that ORF152 bound to calcium-modulating cyclophilin ligand (CAML), a cellular protein that regulates the intracellular Ca^{2+} concentration.
Like that of CAML, expression of ORF152 significantly enhanced the kinetics and amplitudes of the increase in intracellular Ca^{2+} concentration upon stimulation with thapsigargin, a specific and irreversible inhibitor of endoplasmic reticulum Ca^{2+}-APTases. This finding indicates that ORF152 targets cellular CAML to increase the cytosolic Ca^{2+} response.

We also showed that ORF152 acted synergistically with CAML to activate the promoters activator protein 1 (AP-1) and nuclear factor of activated T cells (NFAT), which are related to the calcium-signaling pathway.

To address how ORF152 affects cell differentiation during latent HCMV infection, we induced ORF152 stably into HL-60 cells, which serve as a model for granulocyte-macrophage progenitors. With the addition of ORF152, HL-60 cells showed characteristics of mature macrophage/dendritic cells, such as enhanced expression of cell-surface markers CD80, CD83, and CD86. Maturation of granulocyte-macrophage progenitors is thought to be essential for HCMV reactivation.

Taken together, these results demonstrate that the HCMV latency-associated protein ORF152 induces intracellular calcium concentration, promotes cell differentiation, and acts as key trigger of reactivation from latency.

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Department of Bacteriology

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General Summary

Research projects of our department have focused on: 1) the development of a method for extracting extracellular matrixes from bacterial biofilms and its applicability, 2) high-resolution observation of bacterial biofilms with the atmospheric scanning electron microscope (ASEM), 3) biofilm formation by *Propionibacterium acnes* isolated from pacemakers, 4) high-throughput screening of antibiofilm compounds, 5) analysis of biofilm detachment factor, 6) a simple assay for measuring catalase activity: a visual approach, and 7) analysis of the viable but nonculturable (VBNC) state.

Research Activities

Development of method for extraction of extracellular matrixes from bacterial biofilms and its applicability

Biofilm-forming bacteria embedded in polymeric extracellular matrixes (ECMs) that consist of polysaccharides, proteins, and/or extracellular DNAs acquire high resistance to antimicrobial agents and host immune systems. To understand molecular mechanisms underlying the formation and maintenance of biofilms and to develop therapeutic countermeasures against chronic biofilm-associated infections, we developed an ECM extraction method using high concentrations of sodium chloride and evaluated its applicability. Our method is simple, rapid, inexpensive, and noninvasive and can be used to extract various types of ECM in biofilms formed by diverse bacteria, including Gram-positive *Staphylococcus aureus* and *Staphylococcus epidermidis* and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. Furthermore, this method can be used to clarify molecular mechanisms of biofilm formation and modes of actions of antibiofilm enzymes and drugs.

High-resolution observation of bacterial biofilms with ASEM

The ASEM enables us to observe biological samples in solution at atmospheric pressure. Samples are imaged through a 35-nm-diameter film window made of silicon nitride in the base of the disposable ASEM dish. With ASEM we observed fine structures of bacterial biofilms. Biofilm matrix components (proteins, DNA, polysaccharides, and membrane vesicles) were visualized with heavy-metal labeling, positively or negatively charged nanogold labeling, and immune labeling methods optimized for biofilm samples. These methods could be used to observe biofilms formed by several bacteria, such as *S. aureus*, *S. epidermidis*, *E. coli*, and *Lactococcus lactis*. In conclusion, ASEM is a promising tool to study the fine structures of various bacterial biofilms and their matrix components in solution.

Biofilm formation by P. acnes isolated from pacemaker

P. acnes is a facultative anaerobic Gram-positive commensal bacterium of the human skin, mouth, conjunctiva, and large intestine. *P. acnes* is usually responsible for late chronic infections and rarely causes acute infections related to medical devices. This bacterium has recently been reported to cause infections associated with cardiac devices, breast implants, and prosthetic joints. In this study, 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 using agar plates followed by 16S ribosomal RNA gene sequencing, *P. acnes* was isolated from 8 of 31 devices. An *in vitro* biofilm formation assay showed that glucose is an accelerator for biofilm formation by *P. acnes*. Ultrastructural analysis of *P. acnes* biofilms with the ASEM and transmission electron microscope suggested that the efflux of extracellular substances concomitant with cell lysis contributes to biofilm formation. This work was supported by the Ministry of Education, Culture, Sports, Science and Technology-supported Program for the Strategic Research Foundation at Private Universities, 2012-2016.

High-throughput screening of antibiofilm compounds

A potential strategy for preventing and treating biofilm-associated infections is to use small molecules that inhibit biofilm development. In collaboration with the University of Tokyo, which has a chemically diverse small-molecule library (200,000 compounds), we are now pushing ahead with high-throughput screening to identify the compounds effective against bacterial biofilm development. We have established a crystal violet staining assay of biofilm that is suitable for high-throughput screening. Additionally, we have designed a screening robot system that automates the dispensing of compounds to assay plates, cell culture handling, and activity measurement. To date, 59,600 compounds have been screened with 2 strains of *S. aureus* that form biofilms, and several promising compounds have been identified. Among the compounds, ABC-JK1 showed activity specifically against polysaccharide-dependent biofilm. Several biochemical analyses suggest that ABC-JK1 inhibits extracellular polysaccharide synthesis and thereby inhibits biofilm formation. This work was supported by the Ministry of Education, Culture, Sports, Science and Technology-supported Program for the Strategic Research Foundation at Private Universities, 2012-2016.

Analysis of biofilm detachment factor

The bacteria within the biofilm matrix are protected from the host immune system and from antibiotic attack. Therefore, finding a biofilm-disassembling substance would prove widely useful in medical and industrial applications for preventing or eradicating biofilms. The factor responsible for the detachment effect has a molecular weight < 500 Da and is heat-stable. Culture supernatant was fractionated with gel filtration chromatography and subjected to reverse-phase column chromatography. The flowthrough was subjected to hydrophilic interaction chromatography and eluted with decreasing concentrations of acetonitrile (90% to 0%). The fraction with detachment activity was subjected to metabolome analysis. We are now attempting to identify this factor. The culture supernatant of *S. aureus* also detached the biofilms of *P. aeruginosa,* which is the

causative bacteria of biofilm infections. The molecular weight of the factor responsible for the detachment effect is greater than 10 Da and suggests that there is another detachment factor for *P. aeruginosa* biofilm.

A simple assay for measuring catalase activity: a visual approach

In this study, an assay that combines the ease and simplicity of the qualitative approach for measuring catalase activity was developed. The assay reagents comprised only hydrogen peroxide and Triton X-100. The enzyme-generated oxygen bubbles trapped by Triton X-100 were visualized as foam, whose height was estimated. A calibration plot using the defined unit of catalase activity yielded the best linear fit over a range of 20 to 300 U ($y = 0.3794x - 2.0909$; $r^2 = 0.993$). The precision and reproducibility of the assay at 100 U were 4.6% and 4.8%, respectively. The applicability of the assay for measuring the catalase activity of various samples was assessed using laboratory strains of *E. coli*, catalase-deficient isogenic mutants, clinically isolated Shiga toxin-producing *E. coli*, and human cells. The assay generated reproducible results. In conclusion, this new assay can be used to measure the catalase activity of bacterial isolates and human cells.

Analysis of the VBNC state

Some *E. coli* strains become VBNC under environmental stress conditions and escape detection by conventional culture methods. We showed that a RNA polymerase, sigma S (rpoS)-deficient mutant entered the VBNC state and that the addition of catalase or thiourea to the culture medium resulted in the resuscitation of the bacterium from the VBNC state to a culturable state. These results indicate that the VBNC phenotype is due to the death of σS-deficient stress-sensitive cells induced by oxygen-related radical generation on routine bacterial media under aerobic conditions.

After treatment to induce the VBNC state, wild-type and rpoS-deficient mutant strains were cultured in medium with or without catalase, and DNA breaks labeled with fluorescein isothiocyanate-deoxyuridine triphosphate were analyzed with fluorescence-activated cell sorting. In the wild-type strain, DNA breaks were detected after 1 hour of culture and decreased within 2 hours of culture. On the other hand, the rpoS-deficient mutant strain showed DNA breaks even after 2 hours of culture. The addition of catalase to the medium inhibited DNA breaks in both strains. These results suggest that the VBNC phenotype is due to cell death from the DNA damage induced by oxygen-related radical generation on routine bacterial media.

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Department of Public Health and Environmental Medicine

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

General Summary

1. Our major research projects in the 2013 academic year focused on; 1) the effects of zinc-excess ingestion on blood coagulation in Sprague-Dawley rats; 2) potential mechanisms responsible for tubulointerstitial nephropathy induced by fluoride in rats with unilateral ureteral obstruction; 3) transgenerational effects of maternal arsenic exposure; 4) molecular approaches to cancer chemoprevention with food factors; 5) platelet count and bubble formation after hyperbaric exposure; 6) development of a 14-item health literacy scale; 7) a questionnaire survey of health checkup reports; 8) a questionnaire survey on the quality of life (QOL) of menopausal women; 9) ecological studies of suicide mortality in Japan; 10) the effects of L-carnosine and its zinc complex polaprezinc on pressure ulcer healing; 11) visit-to-visit variability in systolic blood pressure predicts development and progression of diabetic nephropathy, but not retinopathy, in patients with type 2 diabetes; and 12) mental health in the workplace.

Research Activities

Experimental Medicine

1. Effects of zinc-excess ingestion on blood coagulation in Sprague-Dawley rats

Humans are in a zinc-subdeficient state in Japan. Therefore, zinc supplements are commercially available. However, only a few reports of the toxicity of zinc excess have been published. In our previous studies, a hemorrhagic tendency was observed in rats fed a high-zinc diet. Therefore, we focused on blood coagulation observed in rats fed a high-zinc 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 cause renal function to deteriorate further in animals with impaired renal function. In our previous animal experiments, ICR-derived glomerulonephritis mice, which have impaired renal function, were more severely affected by fluoride. In the present study, we used rats with unilateral ureteral obstruction causing tubulointerstitial fibrosis. We examined whether fluoride exacerbates tubulointerstitial nephropathy in rats with unilateral ureteral obstruction.

3. Transgenerational effects of maternal arsenic exposure

Arsenic is a carcinogen in humans. Our recent study has shown that arsenic exposure of maternal C3H mice increases hepatic tumors in male offspring (F1) and grandchildren (F2). Using this model, we investigated the effects of arsenic on the target organs of arsenic, such as the lung, kidney, bladder, and testis.

4. Molecular approaches to cancer chemoprevention with food factors

Carcinogenesis is closely related to lifestyle, including eating habits. For this reason we have tried to develop a preventive approach to cancer using food factors, including phytochemicals and trace elements. We have analyzed the inhibitory effects on the growth of cancer cells and its molecular mechanism. Now, we are focusing on signal transduction pathways related to carcinogenesis, such as mitogen-activated protein kinase/extracellular signal regulated kinase and phosphatidylinositol 3-kinase/Akt pathways. To establish an evidence-based cancer prevention method, we are trying to identify anticancer ingredients in foods, working alone or in combination, and to elucidate their mechanisms of action.

5. Platelet count and bubble formation after hyperbaric exposure

The platelet count is related to the risk of decompression sickness. Several studies have found a decrease in platelet count after decompression. We investigated Doppler bubble detection and platelet count after hyperbaric exposure. The low bubble grade did not change with the platelet count. Our results suggest that the change in platelet count is related to the severity of decompression sickness, not the risk of decompression sickness.

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

1. Development of a 14-item health literacy scale

We developed a generic health literacy measure for Japanese adults which consists of 5 items for functional literacy, 5 items for communicative literacy, and 4 items for critical literacy.

2. A questionnaire survey on health checkup reports

A Web-based survey was conducted among men and women aged 35 to 59 years (n = 424). We evaluated 5 different types of health checkup reports for understanding consumer preferences.

3. A questionnaire survey of quality of life in menopausal women

A Web-based survey was conducted among women aged 45 to 59 years (n = 510). We elucidated the determinants of quality of life in middle-aged Japanese women.

4. Ecological studies of suicide mortality in Japan

We described geographical variations in suicide mortality in Japan and identified, through multilevel analysis, the prefectures and areas that had significant negative and positive contextual effects on suicide risk.

5. Effects of L-carnosine and its zinc complex polaprezinc on the healing of pressure ulcers

We determined the effects of L-carnosine and its zinc complex polaprezinc on the healing of pressure ulcers. The results suggest that L-carnosine and polaprezinc accelerate the healing of pressure ulcers to a similar degree over 4 weeks and that polaprezinc is effective and well tolerated in an 8-week treatment of pressure ulcers.

6. Visit-to-visit variability in systolic blood pressure predicts the development and progression of diabetic nephropathy, but not retinopathy, in patients with type 2 diabetes

We performed a retrospective cohort study and analyzed whether visit-to-visit variability in systolic blood pressure can predict the development and progression of nephropathy and retinopathy independently of mean systolic blood pressure in patients with type 2 dia-

betes.

7. Mental health in the workplace

Mental health in the workplace is increasingly recognized as a serious problem. Several questionnaires have been used in attempts to prevent mental illness in Japan. Concrete questions in questionnaires are important for managing stress in the workplace. The objective of this study was to analyze the association of workers' mental health with job stress and character traits.

Publications

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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 2013 have focused on forensic pathology, DNA analysis, and forensic toxicology. Much of the research was based on forensic practice. The details of our research are described below.

Research Activities

Forensic pathology

1. Analysis of the association between the degeneration of cardiac sympathetic nerves in the subepicardium and unexpected cardiac death

The degeneration of cardiac sympathetic nerves can be found in heart diseases, including ischemic heart disease and cardiomyopathy, and in several diseases with autonomic disorders, including Parkinson disease and diabetes mellitus. However, the relation between the degeneration of cardiac sympathetic nerves in the subepicardium and unexpected cardiac death (UCD) has not been sufficiently examined. Cardiac tissues from forensic autopsy cases were analyzed with immunohistochemical staining for tyrosine hydroxylase and neurofilament. However, the rate of degeneration of sympathetic nerves in the subepicardium of persons with UCD did not differ significantly from that in persons without UCD. Therefore, our results suggest that the degeneration of cardiac sympathetic nerves in the subepicardium does not have a significant effect on UCD, compared with other arrhythmogenic factors.

DNA analysis

1. Identification of war-dead remains with DNA analysis

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

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

We studied a method of DNA extraction from chewing gum. We have investigated the relationship between the amount of extracted DNA and several factors: the weight of chewing gum, the chewing interval, and the number of oral mucosa cells in the chewing gum.

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 (GC/MS), 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, detection of meconin from biological specimens is important in opiate diagnosis. Qualitative and quantitative methods of analyzing meconin with GC/MS were examined.

3. The smoking rate in Japan remains high, and the risk of nicotinic ingestion by smoking cigarettes, including passive smoking, was examined. Nicotine is believed to have enormous effects on embryos, infants, and young children, who have low metabolic capacity. In addition, caffeine can cross the placenta. In specimens obtained at the autopsies of infants, we analyzed nicotine and caffeine with GC/MS to examine nicotine and caffeine exposure to infants. However, neither nicotine nor caffeine was detected in 19 specimens examined.

Radiocarbon analysis

1. Establishment of age estimation

We studied the estimation of date of birth from carbon-14 isolated from dentin. We have investigated a method of specifying the age range from only a single tooth by measuring carbon-14 in incisal (occlusal) and root regions of the dentin separately.

Publications

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Department of Tropical Medicine

Hirotaka Kanuka, *Professor*
Kenji Ishiwata, *Associate Professor*
Masahiro Kumagai, *Assistant Professor*

General Summary

There is a great need to develop novel strategies for parasite control because of the failures of current eradication approaches and the logistical difficulties of implementing them. An interesting aspect of parasitic diseases is that the vector arthropods that transmit the pathogens can mount immune responses against the infection which will kill a large percentage of the parasites. Our group is pursuing research in 4 areas: 1) modification of mosquito vectorial capacity, 2) vector-parasite interactions, 3) immune responses to helminth infection, and 4) the genomics of protozoan parasites.

Research Activities

Early immune responses to chronic infection with a gastrointestinal nematode in mice
Human gastrointestinal (GI) parasitosis is often chronic. However, most murine models of GI parasitic infection terminate after about 2 weeks. In contrast, infection with *Heligmosomoides polygyrus*, a murine GI nematode, persists for about 8 weeks and can serve as a model of chronic infection. To understand the mechanism of chronic infection, early immune responses were examined with flow cytometry and compared with those of acute infection with *Nippostrongylus brasiliensis*. Conventional dendritic cells (DC; $CD11c^{+}B220^{-}$) were analyzed with C-C chemokine receptor type 7 (CCR7), an essential molecule for lymph node recruitment, and MHC class II, an antigen-presenting molecule. Cells positive for CCR7 showed intermediate expression of CD11c and highest expression of class II (DC1: $CCR7^{high}CD11c^{int}$class II^{high}). These DC1 cells showed markedly reduced class II expression from day 4 after *H. polygyrus* infection. On day 8 after *H. polygyrus* infection, the number of CCR7-positive cells, including DC1 cells, was prominently decreased. In the case of *N. brasiliensis* infection, class II expression was reduced on day 5 after infection. Although the number of DC1 cells was decreased on day 8 after *N. brasiliensis* infection, macrophages and newly recruited eosinophils, both of which were CCR7-positive, expressed the same level of class II as did DCs. Concerning $CD4^{+}$ T cells, expression of programmed cell death protein 1, which signals down-regulation, was up-regulated from day 2 after *H. polygyrus* infection. Expression of this protein was slightly up-regulated on day 8 after *N. brasiliensis* infection. These results suggest that reduced ability of antigen presentation and up-regulation of suppressive signals on T cells caused by the parasite are associated with the establishment of chronic GI nematode infections.

Transcriptome analysis of Entamoeba using an ultrafast sequencer
We have been performing transcriptome analysis of *Entamoeba histolytica* and *Ent-*

amoeba invadens, which are parasitic amoebas of humans and reptiles, respectively. Using the oligo-capping method, which guarantees that intact transcripts from the complementary (c) DNA library reach the transcription start site (TSS), we first obtained full-length cDNA sequences. Then, by TSS sequencing analysis, which takes advantage of the oligo-capping method and next-generation sequencing technology, we have determined a massive amount of short (i.e., 36 nt) sequence tags beginning with the TSS. Even though the 5′ untranslated regions were extremely short, the TSSs were not unique to each gene but were clustered. These clusters were distributed unimodally, bimodally, and multimodally, but none showed a flat distribution. We arranged the TSS clusters in order of kurtosis and found that kurtoses of TSS clusters were higher in genes whose TSS tag numbers were larger, i.e., more highly transcribed.

A procedure for permanently stained preparations of cysts of Giardia in stool samples fixed with formalin

Permanently stained preparations of *Giardia* cysts in formalin-fixed stool samples could not be made because: 1) the formalin-fixed stool cannot be smeared on glass; and 2) the formalin-fixed cysts were not stained with solutions used for permanent staining. The first problem was resolved with BD SurePath™ (Becton Dickinson, Franklin Lakes, NJ, USA), a recently developed liquid-based cytology system in which negatively charged cells become attached to positively charged precoated glass slides. Even though the cysts in formalin are not attached to the precoated glass slides, the cysts in water, after being washed with centrifugation in water, are attached. To solve the second problem we applied Kohn's staining with some modifications. Kohn's stain contains a black dye (chlorazol black E) and an alcohol-based fixative called the basic solution. After the basic solution was diluted with water (serially each 10%), the smears were immersed in diluted basic solutions of increasing concentration for more than 10 minutes and then stained with Kohn's stain. As a result, permanently stained preparations were made from formalin-fixed samples: *Giardia* cysts were attached without shrinkage of the cytoplasm, and such structures as the cyst wall, nucleus, karyosome, and flagella were clearly stained.

Molecular dissection of parasitophorous vacuole membrane in malaria liver stage parasites

Malaria is a major global health burden resulting in approximately 1 million deaths each year. The protozoan parasite *Plasmodium* is the causative agent of malaria. This situation has led to attempts to develop novel control and intervention strategies, such as a malaria vaccine. However, these efforts have met with limited success because of the antigenic complexity of the parasite and the differential expression of proteins. Therefore, the next generation of malaria vaccines must include a wide range of activation for the immune response. To clarify the molecular interplay between malaria parasites and the host for future vaccine development, we have focused on malaria liver stage development, especially the parasitophorous vacuole membrane, which is the interface membrane between the parasite and host. Loss-of-function analyses have revealed that protein B9 is specifically expressed in *Plasmodium* liver stages and is important for parasite develop-

ment in the liver. In both rodents and humans *Plasmodium* we show that while *b9* is readily transcribed in sporozoites, B9 protein is present after hepatocyte invasion. Finally we show that B9 is localized on the parasite plasma membrane where it plays a role in establishing and maintaining the parasitophorous vacuole membrane that surrounds the parasite inside the liver cell. On the basis of the structural analyses of the B9 4-cysteine domain and the presence of this domain in several previously identified 6-Cys proteins, we propose that the presence of the 4 positionally conserved cysteine residues are diagnostic for this domain and that B9 belongs to the family of 6-Cys-related proteins. These findings reveal a novel parasite molecular mechanism and suggest malaria liver stage parasites can survive inside host cells.

Antipathogen responses and structural homeostasis maintain midgut wall of malaria vector mosquito

The midgut of disease vectors is the primary and most important physical and immune barrier against pathogens. To clarify the conformation of midgut cells and the function of each cell type, which had been poorly understood, the barrier function of the midgut of mosquitoes (*Anopheles stephensi*) infected with a rodent malaria parasite (*Plasmoidum berghei*) was analyzed with confocal microscopy. When parasites were crossing midgut epithelial cells, the cells invaded by parasites were extruded from the midgut wall, indicating active exclusion of cells damaged by parasites. Meanwhile, in a number of midgut cells containing parasites, phosphorylation of c-Jun N-terminal kinase (JNK), a component of the stress response pathway, was enhanced and showed capsular structures strongly stained with active JNK antibody. This finding suggests a mechanism excluding invaded and damaged cells through the activation of JNK and the encapsulation of pathogens from the midgut wall. Furthermore, midgut stem cells, which undergo mitosis and differentiation, were identified with a 5-ethynyl-2′-deoxyuridine DNA synthesis marker. A number of these midgut stem cells entered mitosis during the 24 hours after blood sucking by the mosquito. Our findings suggest that structural homeostasis mechanisms, such as the exclusion of invaded cells and the division of stem cells, maintain the mosquito midgut wall after pathogen invasion.

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 insect 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 *MyD88*, *Dif1*, and *Dif2*, in addition to JAK/STAT and JNK components, increased the burden of cysticercoids. On the other hand, RNAi-mediated knockdown of immune deficiency pathway components, *dredd* and *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.

Publications

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Department of Laboratory Medicine

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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*. Our study of the measurement of host interleukin 28B single nucleotide polymorphisms for predicting the effects of treatment with interferon for chronic hepatitis C could contribute to advanced medical care. Our new method of high-performance liquid chromatography (HPLC) to determine cholesterol levels of lipoproteins was listed in the insurance publication by the Ministry of Health, Labour and Welfare.

Research Activities

Clinical microbiology

1. Several clinically isolated, previously unidentified bacterial strains were identified though gene sequencing of polymerase chain reaction-amplified 16S ribosomal RNA. We evaluated the status of a methicillin-resistant *Staphylococcus aureus* outbreak in an affiliated hospital through molecular analysis (the phage open reading frame typing method and pulsed-field gel electrophoresis).
2. We performed assays of interleukin 28B single nucleotide polymorphisms to predict the effect of interferon in patients with hepatitis C infection. This assay was useful for deciding whether to perform antiviral therapy, continue treatment, or discontinue treatment.
3. In the field of microbial examination, we studied extraction conditions of multiple blood cultures and the effects of clinical judgments and the analysis of molecular typing of toxin A-negative, toxin B-positive *Clostridium difficile* isolated from nosocomial outbreaks and published the results.

Clinical chemistry

1. Oral tegafur/uracil therapy has been indicated for patients with hepatocellular carcinoma (HCC) and is often used as a single-agent treatment. However, how treatment efficacy is related to fluorouracil (5-FU) metabolic enzymes is unclear. We investigated genetic polymorphisms of the 5-FU metabolic enzymes in Japanese patients with HCC. We examined 2 genetic polymorphisms of the metabolic enzymes cytochrome P450 2A6 and dihydropyrimidine dehydrogenase in 58 Japanese patients with hepatitis C virus-seropositive HCC. To measure efficacy, we investigated genetic polymorphisms of the variable number of tandem repeats of thymidylate synthase and classified the geno-

types as high- or low-expression types. Our results suggested that only 13 of 58 patients with HCC (22.4%) tested would respond positively to treatment with oral tegafur/uracil. Therefore, when administering oral 5-FU to patients with HCC, it is important to consider 3 genetic polymorphisms (cytochrome P450 2A6, dihydropyrimidine dehydrogenase, and thymidylate synthase) associated with 5-FU metabolic enzymes.

2. Our principal research interests are to clarify the pathophysiology of atherosclerosis in relation to impaired lipoprotein metabolism and oxidized low-density lipoprotein and to develop a method of assessing cardiovascular disease risk including the application of our HPLC method to determine cholesterol levels of lipoproteins. We published the following studies.

1) The HPLC method we developed can correlate intermediate-density lipoprotein cholesterol level to Framingham risk score in Japanese men (Int J Cardiol 2013; 168: 3853-8).

2) Pleiotropic effects of hydroxymethyl glutaryl coenzyme A reductase inhibitors (statins) on oxidized lipoproteins are divergent, and pitavastatin can markedly decrease malondialdehyde-low-density lipoprotin/apolipoprotein B while atorvastatin can decrease oxidized high-density lipoprotein/apolipoprotein A1 (Atherosclerosis 2013; 226: 161-4).

In addition, the significant associations of lipoprotein(a)-cholesterol to triglyceride-rich lipoprotein cholesterol, high-density lipoprotein cholesterol and Framingham risk score were found and reported in the accomplishment paper of a Ministry of Education, Culture, Sports, Science and Technology Research Grant. In the meantime, remuneration reimbursement of medical treatment for HPLC lipoprotein analysis was approved by the Ministry of Health, Labour and Welfare.

3. This study was performed to investigate whether the fasting ^{13}C-glucose breath test (FGBT) is useful as a convenient and highly sensitive clinical test for evaluating hepatic insulin resistance. The area under the curve until 360 minutes of the ^{13}C excretion kinetic curve of the FGBT reflects the efficiency of energy production in the liver. The FGBT is a novel glucose metabolism test that can be used conveniently and safely to evaluate the balance of glucose metabolism in the liver. This test has excellent sensitivity for diagnosing alterations in hepatic glucose metabolism, particularly hepatic insulin resistance. (Supported by a Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Strategic Research Foundation at Private Universities, 2011-2015) (performed in collaboration with the National Defense Medical College and the Department of Internal Medicine, The Jikei University)

Clinical hematology

Pathological significance of helper T type 1 lymphocytes in patients with aplastic anemia; Activated T cells play an important role in aplastic anemia (AA). We investigated helper T type 1 (Th1) (interferon γ) and helper T type 2 (Th2) (interleukin 4) lymphocytes in patients with aplastic anemia treated with immunosuppressive therapy. We found that Th1 cells were significantly fewer and that the Th1/Th2 ratio was significantly lower in responders than in nonresponders. These results suggest that measurement of Th1 and Th2 provide important clinical information for the treatment of aplastic anemia.

Clinical pathology

Precisely which cell types, such as hepatic stellate cells (HSCs) in the parenchyma or myofibroblasts in the portal area, contribute most to portal fibrosis, especially in chronic viral hepatitis, remains unclear. Clarifying the characteristics of cells that contribute to portal fibrosis is necessary to determine the mechanism of portal fibrogenesis and to develop a therapeutic target for portal fibrosis. This study examined whether HSCs positive for both lecithin : retinol acytransferase and cellular retinol-binding protein 1 contribute to portal fibrosis in viral hepatitis. This study provided evidence that functional HSCs that coexpress both lecithin : retinol acytransferase and cellular retinol-binding protein 1 and continue to maintain the ability to store vitamin A contribute in part to the development of portal fibrogenesis and parenchymal fibrogenesis in patients with viral hepatitis. (Supported in part by grants from the High Technology Research Center Project for Private University; The Jikei University Research Fund; the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation; the Japan Society for the Promotion of Science Core-to-Core Program, A. Advanced Research Networks; and the Research on the Innovative Development and the Practical Application of New Drugs for Hepatitis B provided by the Ministry of Health, Labour and Welfare of Japan, 2012-13).

Clinical psychiatry

We reported on a patient with epilepsy induced by a specific situation and showed a peculiar clinical course. Furthermore, we examined serum concentrations of new antiepileptic drugs during pregnancy. A study was performed to prevent the recurrence of depression in patients with epilepsy. We plan a clinical study of the management of pregnancy in women with epilepsy.

Clinical physiology

An animal study suggested that prepubertal-onset exercise might help adults maintain long-term body weight reduction and increased energy metabolism after the cessation of exercise.

Addition

We performed studies of the effects of nonspecific materials which gave them to blood data. First we reported the effect of a nonspecific material for measuring tacrolimus with the Affinity Column Mediated Immunoassay (ACMIA) method and studied the effects of a nonspecific material for subsequent measurement of squamous cell carcinoma antigen (SCC).

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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 (0.93, 0.90, and 0.89, respectively) were each higher than for C-reactive protein (0.73, 0.77, and 0.75, respectively). 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. Infliximab is effective at preventing restenosis after the endoscopic balloon dilatation therapy in patients with Crohn's disease

The aim of this study was to evaluate the efficacy and safety of infliximab for preventing restenosis after endoscopic balloon dilatation. We performed the endoscopic balloon dilatation therapy for 14 patients with Crohn's disease. Ten patients had no restenoses when infliximab was administered. Our results suggest that infliximab is useful for preventing restenosis after the endoscopic balloon dilatation therapy in patients with Crohn's disease.

3. Development of optical molecular imaging for gastrointestinal cancer and image-guided 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 recently established a molecular target-specific phototherapy that uses imaging-guided and fluorescence molecular imaging methods in a mouse model of human gastric cancer.

4. Photodynamic surveillance of colitis-associated dysplasia in patients with ulcerative colitis and in mice by visualization following oral 5-aminolevulinic acid sensitization

Photodynamic surveillance with autofluorescent endoscopy after 5-aminolevulinic acid sensitization offers the possibility of detecting low-grade dysplasia in ulcerative colitis by

characteristic fluorescent enhancement. Our data, including our results in mice, suggest this procedure with autofluorescent endoscopy following sensitization by administration of 5-aminolevulinic acid is a promising surveillance method for detecting dysplastic lesions which will be useful for detecting precancerous lesion during ulcerative colitis surveillance.

5. Clinicopathological investigation of predictors of lymph-node metastasis in superficial esophageal squamous cell carcinoma with a focus on evaluation of lymphovascular invasion

Statistical analysis of risk factors for lymph-node metastasis of esophageal superficial carcinoma showed that the strongest risk factor was vascular invasion evaluated with a special staining procedure.

Liver

1. Clinical characteristic of primary biliary cirrhosis and autoimmune antibodies

Antimitochondria antibodies, antinuclear antibodies, and anti-gp 210 antibodies were estimated in a case of primary biliary cirrhosis. The pattern of autoimmune antibodies was compared with the clinical course, outcome, and histological findings.

2. Pathogenesis of minimal hepatic encephalopathy

We found that psychometric testing was a useful method for the early diagnosis of minimal hepatic encephalopathy. We are developing a new nutritional support system for patients with liver cirrhosis.

3. Nutritional imbalance of patients with liver cirrhosis

We 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.

4. 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 and metabolic syndrome. We have tried the possibility of new nutrition supports system with accuracy.

5. Insulin resistance in the liver is critical for the pathogenesis of nonalcoholic steatohepatitis

We applied the fasting ^{13}C-glucose breath test as a noninvasive assessment of hepatic insulin resistance in patients with nonalcoholic steatohepatitis and determined the role of hepatic insulin resistance in the pathogenesis of nonalcoholic steatohepatitis. Additionally, we published reports of a case of hepatic tuberculosis presenting as multiple hepatic masses and a case of peripancreatic tuberculous lymphadenitis diagnosed with endoscopic ultrasound-guided fine-needle aspiration.

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 shows a correlation with prognosis in cases of hepatocellular carcinoma.

6. Treatment response rate of antiviral analogue nucleic acids in chronic hepatitis B virus injection

Resistant viral mutations are an urgent remedial problem in chronic hepatitis B virus (HBV) infection treated with antiviral analogue nucleic acids. The viral gene sequence

analysis and the treatment response were performed. We consider the possibility of a new concurrent therapy for chronic HBV injection.

7. Long-term adefovir-dipivoxil therapy for active chronic HBV infection may provoke chronic renal failure/osteomalacia

We found that the elevation of the serum concentration of bone-specific alkaline phosphatase preceded the elevation of the serum creatinine level and the development of osteomalacia. Therefore, monitoring of bone-specific alkaline phosphatase is useful for predicting and preventing the development of chronic renal failure/osteomalacia.

8. The clinical background of cases of acute HBV 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.

9. Intrahepatic natural killer T-cell kinetics in a mouse model of autoimmune hepatitis

Natural killer T-cell kinetics is a significant reaction in autoimmune hepatitis. Natural killer T-cells and several cytokine profiles were examined in a mouse model of autoimmune hepatitis. Changes in immunoreactions were analyzed in knock-out models.

10. Treatment for chronic hepatitis C virus infection

Predictive factors for the success of treatment with pegylated interferon + ribavirin for chronic infection with genotype 1b hepatitis C virus (HCV) were an increased cholesterol/triglyceride ratio in very low-density lipoprotein and a high level of apolipoproein B-100 along with a major genotype of interleukin 28B. Interleukin 28B single nucleotide polymorphism remained an informative predictor of a sustained virological response. For chronic infection with genotype 2 HCV, prolongation of combination therapy with pegylated interferon + ribavirin from 24 weeks to 36 weeks allowed patients who did not achieve a virological response at 4 weeks to achieve it at 8 weeks.

Pancreas

Wilm's tumor protein 1 (WT1) vaccine in combination with gemcitabine was well tolerated by patients with advanced pancreatic cancer. Delayed-type hypersensitivity-positivity to WT1 peptide and a higher frequency of memory-phenotype WT1-specific cytotoxic T lymphocytes could be useful prognostic markers for survival after combination therapy with gemcitabine and WT1 vaccine.

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General Summary

Our clinical research in 2013 was conducted in the following areas: 1) neurodegenerative disease, 2) cerebrovascular disease, 3) peripheral neuropathy, 4) myasthenia gravis, and 5) epilepsy. We also performed basic research in the following areas: 1) induced pluripotent stem (iPS) cells and 2) lipid metabolism in cerebrovascular disease.

Research Activities

Neurodegenerative disease

1. Cardiovascular autonomic dysfunction in patients with Lewy body diseases, such as Parkinson's disease

Olfactory dysfunction in Parkinson's disease (PD) was significantly related to both cardiac sympathetic and parasympathetic dysfunction, as well as vascular sympathetic dysfunction. As nonmotor symptoms of PD, olfactory dysfunction and autonomic network failure appear to be closely related. Nizatidine was effective for improving the gastrointestinal symptoms in patients with PD of the tremor or indeterminate phenotype but not the posture and gait instability phenotype. Patients with PD for whom nizatidine was effective had milder cardiovascular autonomic dysfunction than did nonresponders.

2. A new device for long-term quantification of hypokinesia and gait bradykinesia in patients with de novo PD

We developed a new device with the MG-M1100 accelerometer (LSI Medience, Japan) to identify gait-induced accelerations based on an algorithm of pattern matching. Five healthy control subjects were asked to walk in sync with metronome beats. The gait cycle was recorded simultaneously with the accelerometer and force plates. No significant difference was observed in the gait cycle recorded with the 2 methods. Twenty-four-hour continuous recordings were obtained from 12 patients with de novo PD and 17 age-matched control subjects. In patients with PD, the number of overall movements was less than in control subjects. Several patients showed a shift of cadence to a bradykinetic rhythm and a narrow range of gait acceleration. The device also detected improvements in gait following treatment with L-DOPA. The results demonstrate that our portable gait ryhthmogram has high sensitivity for the clinically important deficits of hypokinesia and gait bradykinesia.

3. Evaluation of sialorrhea in PD with the Japanese edition of the Sialorrhea Clinical Scale for Parkinson's Disease

We administered the Japanese version of the Sialorrhea Clinical Scale for Parkinson's Disease (J-SCS-PD) questionnaire to 36 patients with PD (14 men and 22 women; mean age, 72.5 ± 8.7 years; mean disease duration, 5.9 ± 2.7 years). This questionnaire consists of 7 items: 1) diurnal sialorrhea, 2) nocturnal sialorrhea, 3) drooling severity, 4) speech impairment, 5) eating impairment, 6) frequency of drooling, and 7) social discomfort. Using the J-SCS-PD, we compared the age, disease duration, Hoehn and Yahr scale, Unified Parkinson's Disease Rating Scale, and clinical subtypes (tremor-dominant, akinetic-rigid, and mixed subtypes). The J-SCS-PD score did not differ significantly between men and women. The J-SCS-PD score correlated with age but not with disease duration or Unified Parkinson's Disease Rating Scale part III score. The mean score was significantly lower for item 5 than for the other items. Among the patients with the 3 clinical subtypes, those with the akinetic-rigid subtype had the highest J-SCS-PD score. Elderly patients had sialorrhea, but eating impairment was of less practical importance than were the other factors.

4. Dysphagia and vocal cord palsy in multiple system atrophy

Dysphagia and vocal cord palsy are not rare symptoms in advanced multiple system atrophy (MSA). Vocal cord palsy is a life-threatening risk factor at the time of percutaneous endoscopic gastrostomy (PEG). We assessed dysphagia and vocal cord palsy with laryngoscopy in cases of MSA. Many patients had vocal cord palsy at the time of PEG. In such patients, PEG should be prepared with noninvasive pressure ventilation therapy.

5. Clinical characteristics of MSA

The manifestations of MSA include movement disorder and autonomic failure. On the basis of phenotype, MSA can be divided into MSA with cerebellar features and MSA with predominant parkinsonism. We examined the clinical picture of MSA in our hospital and how it was related to the results of metaiodobenzylguanidine myocardial scintigraphy, which is an indicator of cardiac sympathetic nerve disorder. The frequency of both dysuria and orthostatic hypotension is high in MSA with cerebellar features, but the frequency of constipation was higher in MSA with predominant parkinsonism. Myocardial scintigraphy with metaiodobenzylguanidine showed denervation in about 17% of cases of MSA. These results suggested that some cases of MSA have same characteristics of Lewy body pathology.

6. Clinical features of progressive supranuclear palsy with predominant cerebellar ataxia

Progressive supranuclear palsy (PSP) is a syndrome that is typically characterized by parkinsonism, postural instability, and cognitive impairment. Our aim was to investigate the clinical characteristics of PSP with predominant cerebellar ataxia. We enrolled 4 patients. Limb and truncal ataxia, early falls, and supranuclear vertical gaze palsy without autonomic dysfunction may predict the diagnosis of PSP with predominant cerebellar ataxia.

Cerebrovascular disease

1. Sonothromolysis for hyperacute stroke with a low-frequency transducer

To increase recanalization rates and improve outcomes after ischemic stroke, ultrasound-enhanced thrombolysis (sonothrombolysis) can improve thrombolytic drug actions.

Recent results indicate that a 2-MHz transducer may be effective for clot lysis in ischemic stroke. Sonothrombolysis is a promising tool for hyperacute ischemic stroke, but its efficacy has been limited for the Asian population because of low ultrasonic penetration. Our aim is to develop a new 500-kHz transducer that improves skull penetration.

2. The effect of vertebral artery hypoplasia on posterior circulation ischemia

The purpose of this study was to evaluate the effect of vertebral artery hypoplasia on posterior circulation ischemia. Subjects were patients with acute ischemic stroke. Patients were categorized by the location of the ischemic stroke on magnetic resonance imaging (MRI).

Results: Of the 129 consecutive patients evaluated, 39 had vertebral artery hypoplasia, and 15 had vertebral artery occlusion. The prevalence of vertebral artery hypoplasia in patients with lesions in only the posterior circulation (44.4%) was significantly higher than that in patients with lesions in only the anterior circulation or with multiple lesions in both both the anterior and posterior circulations (24.7%, $p = 0.034$). Multivariate regression analysis showed that large-artery atherosclerosis, posterior circulation ischemia, and vertebral artery hypoplasia were independent factors related to vertebral artery occlusion. Vertebral artery hypoplasia was an independent factor related to vertebral artery occlusion. Therefore, vertebral artery hypoplasia likely plays a role in posterior circulation ischemia.

3. Characteristics of cerebral microbleeds in Fabry disease

Fabry disease is an X-linked inherited lysosomal storage disorder. Although white matter hyperintensity on MRI has previously been reported in patients with Fabry disease, little is known about cerebral microbleeds. Our aim was to investigate the characteristics of cerebral microbleeds in patients with Fabry disease. Of the 52 patients enrolled, 16 (31%) had cerebral microbleeds. Distinct characteristics of patients with Fabry disease and cerebral microbleeds were male sex, presence of white matter hyperintensity, and kidney dysfunction. These results may help clarify the mechanism of cerebral hemorrhage in Fabry disease.

4. The disappearance of hyperintense vessels on fluid-attenuated inversion recovery predicts a good outcome in patients treated with tissue plasminogen activator

Hyperintense vessels detected with fluid-attenuated inversion recovery MRI in patients with acute ischemic stroke indicate cerebral hypoperfusion. Thus, the disappearance of hyperintense vessels should indicate reperfusion. We investigated serial changes in hyperintense vessels in patients treated with tissue plasminogen activator (t-PA) and compared changes with clinical outcomes. A total of 118 consecutive patients were enrolled, and 52 (44%) were classified as having distal hyperintense vessels. Patients with distal hyperintense vessels had a significantly lower National Institutes of Health Stroke Scale time course ($P<0.001$) and a smaller infarct volume time course ($P<0.001$) compared with patients without distal hyperintense vessels. Multivariate analysis showed that the presence of distal hyperintense vessels was independently associated with good outcomes.

5. Intravenous recombinant t-PA injection should be prepared to treat stroke of in-hospital onset in patients with transient ischemic attack

We are uncertain of the exact clinical outcomes of stroke with in-hospital onset after the admission of patients for transient ischemic attack (TIA), especially for patients treated

with intravenous recombinant t-PA (rt-PA). The aims of our study were to investigate the frequency of TIA in patients with hyperacute stroke treated with intravenous rt-PA and to confirm that patients with TIA possess distinct embolic sources. Of the patients with acute ischemic stroke who had been treated with intravenous rt-PA, 8.5% had had TIA. Distinct embolic sources were detected in 60% of patients with TIA. This percentage was almost as high as that in patients without TIA. We suggested that TIA patients with embolic sources should be treated with rt-PA.

Peripheral neuropathy

1. Intraepidermal nerve fiber density in patients with painful neuropathy

We demonstrated the ultrastructural features of human intraepidermal nerve fibers and surrounding structures in the intercellular space with little shrinkage of keratinocytes. We also evaluated intraepidermal nerve fiber density in patients with painful neuropathy by means of confocal laser scanning microscopy and immunohistochemical staining for protein gene product 9.5.

Myasthenia gravis

1. Estimation of administration period of tacrolimus for patients with myasthenia gravis who have undergone thymectomy

We investigated the administration period of tacrolimus for patients with myasthenia gravis who have undergone thymectomy. We will perform further examinations.

Epilepsy

1. Autonomic symptoms associated with epileptic seizures

Visitor questionnaires and inpatients were investigated in regards to automatic symptoms, such as palpitations, pupillary abnormalities, respiratory changes, perspiration, a desire to urinate, and abdominal symptoms, which tend to be overlooked in the assessment of epilepsy. Common automatic disturbances were nausea and palpitations. The hypothalamic nuclei, the cerebral cortex, and the limbic system comprise a central circuit that affects the autonomic nervous system. The operculum and insular gyrus are domains apparently important for the frontal and temporal lobes since the former are accompanied by emotional change. We speculate that the frontal and temporal lobes and the limbic system are related to autonomic nervous system.

Clinical research

1. Establishment of a high-density lipoprotein functional assay

Either bezafibrate or ethyl icosapentate (EPA) were administered to patients with dyslipidemia. Cholesterol efflux was assessed in the apolipoprotein B-depleted serum of each patient. We used MRI to assess aortic plaques as a clinical outcome. Treatment with bezafibrate significantly increased serum levels of high-density lipoprotein cholesterol and apolipoprotein A-I and decreased triglyceride levels. Cholesterol efflux was greater with bezafibrate therapy than with EPA therapy, although the difference was not statistically significant. Assessment with MRI showed that bezafibrate therapy reduced aortic plaques to a greater extent than did EPA therapy, although the difference was, again, not

statistically significant.

2. Identification of high-density lipoprotein dysfunction in patients with cerebrovascular disease

Cholesterol efflux was assessed in the apolipoprotein B-depleted serum of patients with cerebrovascular disease. Other lipid profiles and the expression of genes were also analyzed. We are recruiting subjects.

3. Comparison of standard and intensive rosuvastatin therapy for 1 year with the primary outcome of aortic plaques evaluated with MRI

This study demonstrated that greater plaque regression was greater with intensive lipid-lowering with rosuvastatin than with standard therapy. We reported this result in the journal *Atherosclerosis* (2014; 232: 31-39).

4. Analysis of cerebral infarction in various types of rodents by means of a model of middle cerebral artery occlusion

In a preliminary study we created rat and mouse models of human adiponectin overexpression and a mouse model of human endothelial lipase overexpression.

5. Characteristics of α-synuclein in PD with iPS cells

Pathological accumulation of misfolded α-synuclein leading to cell dysfunction and cell death plays a central role in the pathogenesis of PD. We analyzed the α-synuclein of iPS cells generated from patients with PD. In 2013, we generated iPS cells from PD and checked how they could be differentiated to neurons by treatment with a reagent. We continue to generate iPS cells from patients with PD and to analyze them.

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Department of Internal Medicine
Division of Nephrology and Hypertension

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General Summary

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

Research Activities

Studies on IgA nephropathy
A randomized clinical trial of steroid pulse therapy with or without tonsillectomy, leading the research group by the Ministry of Health, Labour and Welfare, has been published (Nephrol Dial Transplant, 2014). Steroid pulse therapy combined with tonsillectomy had a significant and independent effect on the disappearance of proteinuria over 12 months. Furthermore, a current multicenter study is expected to provide key insights into immunoglobulin A nephropathy.

Studies of low glomerular density in glomerular diseases and of obesity-related glomerulopathy
Our previous studies have shown that low glomerular density is strongly associated with the prognoses of various glomerular diseases. We have reported that obesity-related glomerulopathy (ORG) reflects obesity-induced renal injury and also accompanies a state of renal mass reduction. Both of these factors appear to synergistically contribute to the development of ORG (Nephrol Dial Transplant, 2013). Moreover, we investigated the characteristics of a cohort of Japanese patients with ORG and compared them with cohorts in other countries (Clin Exp Nephrol, 2013). Furthermore, collaborative research to estimate the number of nephrons in Japanese subjects is in progress.

Studies of glomerular epithelial cells (podocytes)
Transgenic mice (NEP25) express human CD25 selectively on podocytes, and injection of a human CD25-targeted recombinant immunotoxin permits selective injury to podocytes. We investigated the mechanisms of podocyte regeneration after glomerular injury using this model (Nephrol Dial Transplant, 2014). Furthermore, we investigated the effect of oxidative stress on podocyte injury, focusing on the Keap1-Nrf2 system, a mas-

ter regulator of the antioxidant response. We demonstrated that Nrf2 activation by genetic Keap1 knockdown attenuated podocyte injury (Nephrol Dial Transplant, 2014).

Studies of CKD mineral and bone disorders

To determine whether normal parathyroid cells have a similar extracellular Ca^{2+} entry system, cells were isolated from normal human parathyroid glands. Normal human parathyroid cells express a dihydropyridine-sensitive Ca^{2+}entry system that may be involved in the $[Ca^{2+}]$o-induced change in $[Ca^{2+}]$i. In a clinical study we clarified that ferric citrate hydrate, a novel iron-based phosphate binder, decreased concentrations of fibroblast growth factor 23.

Studies of peritoneal dialysis

Encapsulating peritoneal sclerosis (EPS) is a serious complication in patients receiving long-term peritoneal dialysis. In a retrospective, observational study, we found that the dialysate-to-plasma ratio of creatinine and the duration of peritonitis were independently associated with EPS. Thus, we conclude that earlier treatment to promote an early recovery from peritoneal dialysis-associated peritonitis could be critical in preventing EPS.

Study of renal transplantation

We examined graft survival in patients who had undergone renal transplantation to determine the significance of caveolin 1 immunoreactivities in the peritubular capillaries.
We compared clinicopathological factors between mismatch and controls. We found that both glomerular hypertrophy and proteinurea were more common in the mismatch group than in the control group.

Studies of polycystic kidney disease

The goals of our research into autosomal dominant polycystic kidney disease (ADPKD) are to investigate the pathophysiology of ADPKD and to develop new therapies. We have reported a functional assay of *PKD1*/*PKD2* genes, described the mechanism of cyst formation, and performed a therapeutic investigation of in-vitro cysts from patients with ADPKD. Currently, research regarding the genetic analysis and the genetic counseling of patients with ADPKD is in progress.

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

We examined the mechanism of the renal protective effects of azilsartan in a rat model of renal failure. Daily urinary sodium excretion was not significant; however, azilsartan tended to suppress plasma aldosterone, daily urinary protein, and norepinephrine excretion compared with vehicle. Our findings suggest that the renal protective effects of azilsartan are due in part to the suppression of aldosterone and the sympathetic nervous system.

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

We examined the relationship between central blood pressure (CBP) and the renin-angio-

tensin-aldosterone system in patients with primary aldosteronism and essential hypertension. The gap between 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.

Association of serum uric acid and observation of kidney tissue in patients with CKD
In patients with CKD, we examined how serum uric acid is associated with pathologic changes in kidney tissue. Elevated serum uric acid levels were strongly associated with interstitial fibrosis and tubular atrophy and weakly associated with hyalinizing arterioles. An increased serum level of uric acid was not associated with glomerular global sclerosis or increased thickness of the arcuate and interlobular arteries. Hyperuricemia is closely associated with the arterioles and tubulointerstitial lesions in patients with CKD.

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Department of Internal Medicine
Division of Rheumatology

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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 quikcly 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 collagen-induced arthritis (CIA) mice. 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

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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 diseases, into a database. Using this precise database, we have been performing a study comparing risk factors, clinical outcomes, and others. In addition, we have participated in nationwide clinical studies, such as J-DESsERT (The Japan-Drug Eluting Stents Evaluation; a Randomized Trial), J-LESSON (Japan Unprotected Left Main Coronary Artery Disease Percutaneous Coronary Intervention Strategy On New Generation Stents), RESET (Randomized Evaluation of Sirolimus-eluting versus Everolimus-eluting stent Trial), NEXT (NOBORI Biolimus-Eluting versus XIENCE/PROMUS Everolimus-eluting Stent Trial), 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), NIPPON (Nobori Dual Antiplatelet Therapy as Appropriate Duration), and OPERA (Optimal Duration of DAPT Following Treatment With Endeavor [Zotarolimus-eluting Stent] in Real-world Japanese Patients). Most of these studies have investigated treatment with drug-eluting stents and the diagnosis of coronary spasm, which is closely related to the etiology of ischemic heart disease.

Arrhythmia Research Group
We have a special team for the management of patients with arrhythmic diseases. We focus on the curative treatment of atrial fibrillation through catheter ablation. All types of arrhythmias, including tachycardia and bradycardia, are treated. We have drawn fully upon the strength of new devices and pioneering approaches and have actively reported new findings.

Heart Failure Research Group
In regard to heart failure research, we have been especially assessing data related to plasma levels of B-type natriuretic peptide (BNP), which is an sensitive marker of heart failure, and been participating in multicenter research on standard values that will be of use in clinical practice. In addition, we have reported in detail on plasma BNP levels in patients with acute heart failure before and after admission to the hospital and are now assessing clinical data regarding the relationship between plasma BNP levels and obesity.

Imaging Research Group
Multidetector (row) computed tomography has become a reliable method for detecting coronary arterial organic stenosis. We have been investigating the possibility that a change in coronary arterial tone can also be detected with repeated multidetector (row) computed tomography. We are also studying new approaches of echocardiography.

Molecular Biology Research Group
In myocardial metabolism, we have investigated an association between cellular damage and intracellular ion kinetics. We have also studied the decrease in the serum potassium concentration during acute coronary syndrome. We hypothesized that aldosterone is involved in a cardioprotective mechanism in the acute phase of ischemia and in abrupt cellular damage. We found a previously unknown nongenomic action of aldosterone by using cultured neonatal rat cardiomyocytes and the Langendorff system. We are now focusing on the mechanisms of changes in energy metabolism in the failing heart.

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 studied the relationship between Ca^{2+} leak and altered Ca^{2+} uptake in sarcoplasmic reticulum using a genetically engineered mouse heart. We have also reported the role of thrombin on cardiac disease, leading to experiments using a mouse model of dilated cardiomyopathy.

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General Summary

Physicians should practice patient-oriented medicine based on the concept of evidence-based medicine, which consists of research evidence, clinical expertise, and patients' preferences. To accomplish this goal, we encourage the members of our staff to do basic and clinical research. Areas of research include diabetes, metabolism, and endocrinology.

Research Activities

Epidemiology and evidence-based medicine
Several clinical trials of the treatment of type 2 diabetes using continuous glucose monitoring (CGM) are under way. The relationship between glucose fluctuation and diabetic complications as well as glucose fluctuations in patients with drug-naïve type 2 diabetes is also studied using the data from CGM.
A nationwide epidemiologic study of mortality in approximately 3,500 patients with type 1 diabetes was started in 1986 and has continued to provide much information about the prognosis of Japanese children with type 1 diabetes. A population-based study of childhood obesity and insulin resistance, diabetes in the elderly, and genetic factors has also continued in Niigata Prefecture.

Molecular diabetology
Objective: Spontaneous hypoglycemia occurs owing to several causes with different patterns of hypoglycemia and hormone responsiveness. The aim of this study was to identify gene mutations in a family with spontaneous hypoglycemia by focusing on candidate genes and evaluating metabolism and hormone status.
Methods: The metabolic state was observed with CGM during the starvation test in the proband. Genomic DNA from peripheral blood was sequenced directly to identify gene mutations.
Results: The proband was a 34-year-old woman who was admitted to our university hospital because of severe hypoglycemia and metabolic acidosis associated with diarrhea and loss of appetite. She had had hypoglycemia-like episodes, especially when fasting, since the age of 1 year. In the starvation test, CGM clearly demonstrated no hypoglycemia until 29 hours. However, once hypoglycemia occurred at 29 hours, it persisted even

after the induction of glucagon and the suppression of insulin secretion. These findings strongly suggest that a glyconeogenic enzyme is inactive. Therefore, we focused on key glyconeogenic enzymes, including fructose-1,6-bisphatase (FBP1), phosphoenolpyruvate carboxykinase 1, and pyruvate kinase. The sequencing of these enzymes revealed that the proband and her brother, who had similar hypoglycemia-like episodes, share a mutant genotype of compound heterozygosity for *FBP1* (G164S/F194S), in which homozygotes of each allele had been reported as a responsible mutation for the phenotype.
Conclusion: Observation of hypoglycemia with CGM and hormone responsiveness in a patient with hypoglycemia permitted a focus on candidate genes and enabled identification of *FBP1* mutations.

Insulin resistance and obesity
A series of basic research studies of insulin resistance were performed in Otsuka Long-Evans Tokushima Fatty rats. The effects of a new oral hypoglycemic agent (a dipeptidyl peptidase IV inhibitor) on insulin resistance were investigated.

Dietary therapy
A highly monounsaturated enteral formula more effectively suppressed postprandial hyperglycemia without causing exaggerated insulin secretion compared with a high-carbohydrate enteral formula in patients with type 2 diabetes and healthy subjects. In patients with type 2 diabetes, tube feeding with a highly monounsaturated eternal formula was shown with CGM to suppress postprandial hyperglycemia and to reduce 24-hour glycemic variations to greater extent than did a high-carbohydrate eternal formula, even if carbohydrate nutrients had been adjusted for a low glycemic index.

Diabetic vascular complications
Diabetic complications are major sources of morbidity and mortality in patients with diabetes and an economic burden on societies worldwide. A greater understanding of the molecular targets that regulate both microangiopathy and macroangiopathy could lead to novel therapeutic strategies against diabetic complications. The Rho guanosine triphosphatases and their downstream effectors, Rho-associated kinases (ROCKs), have been implicated as regulators of the actin cytoskeleton. Because changes in the actin cytoskeleton are associated with vascular function, recent studies have revealed that ROCKs play a pivotal role in cardiovascular diseases, such as atherosclerosis, and in vascular remodeling. Accumulating evidence from animal models of diabetes shows that ROCK activity is increased in the kidney, retina, and vessels. Studies using pharmacological inhibition and genetic deletion of ROCKs have demonstrated that ROCK inhibition suppresses diabetic nephropathy by attenuating the excessive production of extracellular matrix induced by diabetes and slows the development of glomerular sclerosis and interstitial fibrosis. Given this background, we investigated the mechanism by which Rho-kinase promotes diabetic nephropathy. We found that ROCK inhibitor attenuates chemokine production and macrophage infiltration in mesangial cells. We conclude that ROCK is an important therapeutic target against diabetic complications.

Endocrinology

To identify and isolate stem-like cells in human pituitary adenomas, we focused on the expression of CD133, which is a tumor stem cell marker in brain tumors, and examined the differences between CD133-positive cells and CD133-negative cells indicating stem properties.

The 12-lipoxygenase pathway may play a role in the pathogenesis of diabetic cardiomyopathy. Therefore, the role of the 12-lipoxygenase pathway in cardiomyopathy was examined in a rat model of diabetic cardiomyopathy and in an in-vitro study with a primary cardiomocyte culture system.

Previous studies have shown that the secretion of adrenocorticotropic hormone is increased in the hearts of patients with hypertension, indicating that adrenocorticotropic hormone may be involved in the pathophysiology of cardiovascular diseases. Recently, pro-opiomelanocortin messenger RNA has been shown to be expressed in the murine heart. Therefore, we designed a study using HL-1 cardiomyocytes to clarify the pathophysiological role of pro-opiomelanocortin.

In patients with hyperaldosteronism, Ca blockers (type T and type N) reduce levels of aldosterone.

In patients with hypertension and type 2 diabetes, fluctuations of glucose and systolic blood pressure were found to be related and to be associated with the development of arteriosclerosis.

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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 2013.

Research Activities

Leukemias
Many patients with previously untreated hematological disorders have been referred to our department. The disorders in 2013 included acute myeloid leukemia (AML), 23 cases; acute lymphoblastic leukemia (ALL), 6 cases; chronic myeloid leukemia (CML), 1 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 2013 were as follows: AML-209-GS, AML209-KIT, JALSG-ALL-CS-12, JALSG-CS-11, JALSG AML209-FLT3-SCT Study (AML209-FLT3-SCT), 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. We also participated in several cooperative group studies and pilot studies: Aged Double-7 (newly diagnosed AML in the elderly: phase II), VEGA (MDS: phase II), a study of nilotinib (refractory CML: phase I/II), and a study of dasatinib (refractory CML: phase I/II).

Lymphomas
In 2013 we registered 77 patients with newly diagnosed non-Hodgkin's lymphoma and 7 patients with 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 10 patients with newly diagnosed multiple myeloma in 2013. 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 last year. 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-positive 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.

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.

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Department of Internal Medicine
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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 and manuscript has been submitted. We are also completed the study investigating etiologies of acute exacerbation of bronchial asthma and COPD in adults by real-time polymerase chain reaction. Concerning basic research, we are investigating lung homeostasis, especially aging, apoptosis, necrosis, and senescence. We also interested in autophagy in the pathogenesis of lung diseases.

Research Activities

Cellular senescence and autophagy in COPD

COPD is caused by the noxious effects of tobacco smoke, which leads to airway epithelial cell injury and the induction of phenotypic changes, such as squamous metaplasia and cellular senescence, which are assumed to be part of the adaptive response to toxic components, such as reactive oxygen species (ROS). 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 apopto-

sis and cellular senescence. Accelerated cellular senescence is implicated in the pathogenesis 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).

Cellular senescence and autophagy in idiopathic pulmonary fibrosis

Aberrant re-epithelialization with bronchial epithelial cells is a prominent pathologic finding in idiopathic pulmonary fibrosis (IPF) and is implicated in abnormal epithelial-mesenchymal interactions. Recent studies have shown that senescence is a risk factor for the development of IPF. Among the SIRT 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 β1 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).

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 PCR for respiratory samples was used. We prospectively enrolled adult patients with AEBA who satisfied 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 real-time 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 real-time 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.

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Yamakawa H, Takayanagi N[1], Miyahara Y[1], Ishiguro T[1], Kanauchi T[1], Hoshi T[1], Yanagisawa T[1], Sugita Y[1] ([1]Saitama Cardiovasc Respirat Ctr). Prognostic factors and radiographic outcomes of nontuberculous mycobacterial lung disease in rheumatoid arthritis. *J Rheumatol.* 2013; **40:** 1307-15.

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Reviews and Books

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Department of Internal Medicine
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Research Activities

Division of General Medicine, The Jikei University Hospital

We are attempting to compile a database of our medical examinations and treatments during primary care in outpatient units. The data and information of every outpatient are collected from forms of our own design after being filled out by physicians. The data and information include reason for visiting, symptoms and complains, whether the patient had consulted other physicians, the primary diagnosis, examinations, and care. This year, the most frequent reasons for consultation were abdominal pain, cough, and pyrexia. The most frequent initial diagnose 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 health care, including community health care. 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 case reports associated with endocrine diseases: pituitary disease, adrenal disease, thyroid disease, and electrolyte abnormality.

We planned a study of thiamine (vitamin B1) metabolism. We measured serum thiamine levels in many outpatients and inpatients with pretibial pitting edema and sensory disturbance of the lower limbs. Patients were divided into 2 groups on the basis of the serum thiamine level. We are going to compare the 2 groups in terms of insulin secretion and peripheral neuropathy leading to paralysis, weakness, lower limb paresthesia, and wasting of muscle.

Division of General Medicine, The Jikei University Daisan Hospital

1. Study of nutritional support in elderly patients

We studied nutritional support in elderly patients. We found that the intake of fewer calories and less fluid improves prognoses and reduces the pain of elderly patients. The prevention of catabolism due to inflammation is more important than sufficient administration of nutrition.

2. Study of polymyalgia rheumatica

We found that patients with low levels of C-reactive protein and matrix metalloproteinase 3 require lower doses of prednisolone. We can guide remission by the same dose of

prednisolone in cases of recurrence.

3. 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.

4. 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. New markers are needed for the early diagnosis of sepsis.

Division of General Medicine, The Jikei University Kashiwa Hospital

Our research in The Jikei Kashiwa Hospital consists of 4 parts. The first part is to investigate the role of general medicine on environmental health achievement in the region, especially in Kashiwa City; we also participated again in this year in the development of a local health care system in Kashiwa as part of a committee on local governance. The second part is to develop educational tasks for teaching medical students and junior physicians. The third part is to establish 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.

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Research Activities

Psychopathology, psychotherapy and child study group

We have performed research in psychotherapy, psychopathology, and child psychiatry. We have investigated the care systems for developmental disorders in the psychiatry unit. We began a study of the attention problems of patients with developmental disorders or psychotic disorders. This study investigated the quality of attention in autism-spectrum disorders, but when many tasks were added, the quality of attention tended to decrease. In psychotherapy, we attempted to develop prototypes of dialectical behavior therapy for Japanese patients, diary therapy, and the self-psychological psychotherapeutic approach, which maintains the self-esteem of patients with developmental disorders and develops cognitive functions. We have been investigating the possibility of a psychoanalytic approach for development disorders.

Our social psychiatry team investigated background factors related to depression in white-collar workers. This study suggested that male workers exhibiting perfectionism tend to undertake too much work and to become exhausted when trying to cope with complex human relationships in the workplace. Female workers having the double burden of family commitment and perfectionism tended to be isolated in terms of personal relationships, leading to exhaustion both inside and outside the workplace.

Morita therapy group

In cooperation with psychotherapists of other schools, such as cognitive-behavioral therapy and psychoanalysis, we are developing programs and materials to train young psychiatrists in 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 the field of palliative medicine. In addition, studies continuing this year examined the subtypes of obsessive-compulsive disorder, the psychopathology of social anxiety disorders, and factors in the recovery of patients with depression through inpatient Morita therapy.

Psychopharmacology group

In basic research, we performed the following studies in rodents: 1) effect of a novel psychotropic agent on monoamine neurotransmission using microdialysis and radioimmuno-

assay techniques, 2) the formation mechanism of drug addiction, 3) the neural basis of addiction-related impulsivity, and 4) the development of a novel anticraving agent (the latter 3 studies were performed in collaboration with the NTT Communication Science Laboratories and the Department of Psychology, Senshu University). In clinical research, we have performed the following studies in humans: 1) the effect of second-generation antipsychotics on anxiety and stress-related disorders, 2) the effect of antipsychotics on dopaminergic neurotransmission using positron emission tomography (in collaboration with the National Institute of Radiological Sciences), 3) exploratory research on novel blood biomarkers for mood disorders (in collaboration with the Department of Virology, The Jikei University School of Medicine), and 4) the effect of modified electroconvulsive therapy on regulatory factors for gene expression, and 5) the symptoms of 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 revealed that anxiety in Alzheimer's disease was correlated with hyperperfusion in the bilateral anterior cingulate cortices and a reduction in the gray matter volume in the right precuneus and inferior parietal lobule. Another study revealed that attention deficits, executive dysfunction, and working memory are observed in patients with late-life somatization disorders. The finding that attention deficits are not correlated with the severity of symptoms suggests that attention deficits are associated with the appearance of symptoms. Executive dysfunction and working memory might be associated with the severity of symptoms. We are planning to investigate further effects of these symptoms on the daily lives of patients and the burden on caregivers.

General hospital psychiatry

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 cancer. We are now focusing on risk factors for postoperative delirium in patients with digestive tract cancers.

Clinical electroencephalography group

We reported a case of epilepsy that was induced by a specific situation and showed a peculiar clinical course. Furthermore, we examined the serum concentrations of new antiepileptic drugs during the pregnancy of patients with epilepsy. A study was performed to prevent the recurrence of depression in patients with epilepsy. We are planning a study of the management of epilepsy in pregnant women.

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 Mr. Tsunemoto Suzuki to a clinical conference and studied "autogenic training" and its clinical use. Furthermore, we trained graduate students of a clinical psychological course.

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General Summary

We have 10 subspecialty research groups, which are 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.

Research Activities

Inherited metabolic disease group
The achievements of our group this year are as follows.
1. We developed a novel glycosaminoglycan (GAG) assay method to identify specifically accumulated GAG in a mouse model of mucopoloysaccharidosis type II (MPS II). Using this method, we showed that brain GAG was reduced by lentiviral vector-mediated hematopoietic stem cell gene therapy in MPS II mice.
2. We analyzed the mechanism of cardiomyocyte hypertrophy in Pompe disease using induced pluripotent stem cells. In addition, anti-human CD3 antibody (otelixizumab) induced immune tolerance against infused enzyme in enzyme replacement therapy for Pompe disease in transgenic mice expressing human CD3.
3. Using multiplex ligation-dependent probe amplification and cDNA analysis, we found 4 novel mutations in the α-galactosidase A gene from patients with Fabry disease.
4. We performed genetic diagnoses with a comparative genomic hybridization array and exome analysis in patients with congenital anomalies and intellectual disabilities.

Neurology group
Our research aims to clarify the clinical, genetic, and molecular pathophysiological aspects of childhood genetic epilepsies, especially intractable early-onset epilepsies, and to develop more effective treatments. We are focusing on 2 monogenic epilepsies, Dravet syndrome (mostly caused by defects of sodium channel, voltage-gated, type I, alpha subunit [SCN1A]) and protocadherin 19 (PCDH19)-related female epilepsy. Regarding Dravet syndrome, we have successfully generated a new research platform using patient-

derived induced pluripotent stem cells and have identified a functional vulnerability in the patient-derived neurons.
We investigated visiospatial disturbances in 103 children more than 1 year after onset of acute encephalopathy.

Nephrology group
Yamada and his colleague analyzed the gene expression profiles and differentiation capabilities of bone marrow- and adipose-derived mesenchymal stem cells in a rat model of chronic kidney disease (CKD). The study revealed that uremic toxin in CKD rats had a small effect on the gene expression and differentiation of mesenchymal stem cells. Using data from a nationwide survey, Hirano studied the effect of birth weight on the development of CKD in childhood and showed a strong association between low birth weight and the development of CKD in children.

Infectious diseases and Immunologic Disorders group
We studied the identification of causative pathogens by means of multiplex polymerase chain reaction techniques in pediatric inflammatory diseases and respiratory infectious diseases. We also investigated the pathogenesis of and new treatments for chronic granulomatous disease (CGD) at the Department of Human Genetics of the National Research Institute for Child Health and Development. We found significantly lower incidences of *Bacteroides* and *Clostridium* in patients who had CGD with colitis than in patients who had CGD without colitis. This result suggests that a compositional change of the intestinal microbiota is useful for the early diagnosis of CGD colitis. Furthermore, we reported that thalidomide was effective for treating bowel inflammation in patients with CGD and did not cause progression of fungal or bacterial infections. Thalidomide is an efficacious therapeutic option for patients with CGD. We are planning to attempt gene therapy for certain patients with CGD in the near future.

Hematology and Oncology group
We investigated mutations of exons of 409 tumor-suppressor genes and oncogenes most frequently cited and most frequently mutated in the malignancies associated with congenital anomalies by using the Ion AmpliSeq™ Comprehensive Cancer Panel (Life Technologies, Carlsbad, CA, USA). Moreover, we have a started a project to use whole-exome analysis to identify the genes causing undiagnosed congenital anomaly syndrome.
We performed a cross-sectional survey of pain management during bone marrow aspiration and biopsy and presented the results to institutions belonging to the Tokyo Children's Cancer Study Group. Seeking to acquire relevant pharmacological knowledge and share the information with palliative care teams, we established *Guide to the Pharmacological Management of Symptoms in Children with Cancer*.

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

1. Right heart failure and peroxisomal proliferator-activated receptor gamma
2. The effects of telmisaltan in heart failure
3. The effects of bisoprolol in right heart failure
4. The effects of carperitide in monocrotaline-induced pulmonary hypertension
5. Assessment of cardiopulmonary function in metabolic heart disease
6. Urocoltin and angiopoetin evaluation in congenital heart disease
7. Early diagnosis of renal dysfunction in patients with congenital heart disease
8. Problems of the Fontan operation, with a focus on operative methods
9. Our research on right heart failure and peroxisomal proliferator-activated receptor gamma recieved the Young Investigator Award at the annual meeting of the Association for European Paediatric Cardiology in 2014.

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 organized and performed the following multicenter clinical studies: the PET study (The Preventive Effect of Tulobuterol Patch for the Long-Term Management of Infantile Asthma study), the PARG study (Pediatric Asthma Research for Guideline Update: Add-on use of tulobuterol patch on unstable asthma treated with leukotriene receptor antagonist), the CIT study (A Comparison of Continuous Inhalation Treatment with Salbutamol and Isoproterenol for Severe Pediatric Bronchial Asthma: A multicenter, double-blind, randomized study), the OSCAR study (Optimal Stepdown Therapy for Controlled Pediatric Asthma Responded to SFC), and the ORIMA study (Effect of Oral Immunotherapy in Preschool Children with Milk Allergy study). We have just started 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

We used immunohistochemical methods to analyze the expression of urocortin 2, urocortin 3, and inflammatory cytokines in the brain in 2 rat models of surgical left ventricular heart strain (high and low left ventricular pressure). Furthermore, we performed tail-suspension tests for these rats and analyzed behavior patterns.

We identified 3 novel mutations of the *SLC16A2* gene in 3 patients with suspected deficiency of monocarboxylate transporter 8 and performed clinical investigations. We found endocrinological abnormalities in all 3 patients.

We studied the efficacy of growth hormone treatment in children with small for gestational age short stature born with extremely low birth weight. The method of individually adjusting doses of growth hormone was considered the best way of using growth hormone to treat these patients.

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Department of Dermatology

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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, and ustekinumab, 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 Janus kinase 1/3 inhibitor.

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 the Severity Scoring of Atopic

Dermatitis Index and the 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 a topical phosphodiesterase 4 inhibitor 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 the PCR has regularly been performed for bowenoid papulosis and rare viral warts. Five-percent imiquimod cream is now 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 ultrapulse 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 phototherapy equipment, such as the 308-nm excimer lamp, 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®, 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 cytokines 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

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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. Ovarian serous borderline tumors: Magnetic resonance imaging findings

We reviewed magnetic resonance imaging (MRI) findings of 9 patients in whom serous borderline tumors (SBTs) were diagnosed. The SBTs were either cystic tumors with papillary projections or exophytic papillary projections. Papillary projection, which is a papillary architecture showing high signal intensity on T2-weighted images with internal branching showing low signal intensity on T2-weighted images, increases the possibility of SBTs.

4. Early CT findings of clinically amyopathic dermatomyositis

Rapidly progressive interstitial pneumonia associated with clinically amyopathic dermatomyositis easily causes respiratory failure and is often fatal. Immediate treatment is critical for improving prognoses. We reviewed high-resolution CT findings and clinical conditions on admission in 5 cases of rapidly progressive interstitial pneumonia associated with clinically amyopathic dermatomyositis to clarify useful imaging findings in the early stage of this disease.

5. Chest CT findings of immunoglobulin G4-related disease

Immunoglobulin G4-related disease is a multiorgan disorder that can involve the lungs. We reviewed 25 cases of immunoglobulin G4-related thoracic abnormality from our archives from April 2011 through March 2014 to identify characteristic CT features.

6. Detection of insufficiency fractures of the pelvic bone with tomosynthesis

This study evaluated the diagnostic performance of tomosynthesis in the depiction of insufficiency fractures of the pelvic bone with use of MRI and CT as references and tested

whether tomosynthesis is more effective than X-rays for detecting such lesions.

7. Morphological and hemodynamic evaluation of the cardiovascular system with dual-source CT

Detailed anatomic features of normal cardiac structures, such as the foramen ovale, and hemodynamic information in cases of complex congenital cardiac anomalies were evaluated with a dual-source CT unit and an ultrahigh-speed scan technique.

8. MRI evaluation of the therapeutic effects of biological agents in psoriatic arthropathy

In patients with psoriatic arthropathy (PsA), MRI was performed before and after the start of the treatment, and the presence or absence of enthesitis, synovitis, bone marrow edema, and bone erosion was evaluated. In patients with active PsA, the contrast-enhancement 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 PsA.

9. Evaluation of bone marrow signal abnormalities at the cruciate ligament enthesis

Changes in bone marrow signals at the tibial cruciate ligament enthesis are frequently observed. Tubular lesions were observed in approximately 30% of cases, and cystic lesions were observed in 10%. Tubular lesions may represent vascular structures penetrating from the surface of the cruciate ligament to the tibia. Furthermore, a correlation was observed between cystic lesions and the severity of osteoarthritis. This outcome suggests that mechanical stress to an enthesis causes tubular structures to be modified into cystic lesions.

Division of Ultrasound

1. Clinical usefulness of sonographic contrast agent in breast tumors

The efficacy and safety of ultrasonography with contrast enhancement using Sonazoid microbubbles (Daiichi Sankyo Co., Ltd., Tokyo) for the diagnosis of breast lesions were analyzed. Ultrasonography with contrast enhancement had significantly better diagnostic accuracy and specificity than did noncontrast studies and caused no serious adverse reactions.

2. Power Doppler ultrasonography for evaluating the activity of rheumatoid arthritis

Power Doppler ultrasonography was performed in the right and left 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 of the scores 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 on 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 in 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. Efficacy and safety of our new technique of ipsilateral percutaneous transhepatic portal vein embolization
Percutaneous transhepatic portal vein embolization was performed to increase the volume of the left hepatic lobe before hepatic resection in 8 patients. With ultrasonic guidance, a balloon catheter was introduced into the right portal vein. A gelatin sponge was injected via the sheath while the right portal vein was occluded with a balloon. Two weeks after the procedure the volume of the left hepatic lobe was assessed with either CT or scintigraphy. The volume of the future liver remnant was increased by 46.5% ± 31.5%. There was no complications or progressive liver insufficiency after embolization or resection.

Division of Radiation Therapy
1. Clinical study of radiosensitization therapy using by topical injection of low concentration hydrogen peroxide and hyaluronate
Kochi oxydol‐radiation therapy for unresectable carcinomas (KORTUC) is an enzyme‐targeting radiosensitization treatment that uses a radiosensitizer containing a low concentration of hydrogen peroxide with or without sodium hyaluronate. Hydrogen peroxide inactivates peroxidase/catalase in the tumor tissue.
Most locally advanced neoplasms contain many hypoxic cancer cells or large amounts of antioxidative enzymes and are, therefore, resistant to low linear energy transfer radiation. KORTUC I uses a hydrogen peroxide solution‐soaked gauze that covers superficially exposed tumors. With KORTUC II, the radiosensitizer is injected into the tumor tissue under ultrasonographic or CT guidance. Ten patients with locally advanced malignant neoplasms entered our clinical trial and were treated with KORTUC I or II.
2. Radical radiotherapy for prostate cancer
There are various treatment options for prostate cancer. In radiotherapy for prostate cancer, progress has recently been made in ultrahypofractionation. Stereotactic body radiotherapy (SBRT) has attracted considerable attention as a modality 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 the efficacy of locoregional control and overall survival after concurrent chemoradiotherapy with fluorouracil and cisplatin for DCF-refractory esophageal cancer.

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Department of Surgery
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General Summary

We researchers in the academic environment must be familiar with statistical tests, including linear and logistic regression analyses.
Although a large number of basic and clinical studies were published by our division from April 2013 through March 2014, we must make greater efforts to publish articles in journals with high impact factors. Thomas Wakley, the founder of *The Lancet*, said, "A lancet can be an arched window to let in the light or it can be a sharp surgical instrument to cut out the dross and I intend to use it in both senses."

Research Activities

Upper gastrointestinal surgery
We are attempting to evaluate the pathogenesis of primary esophageal motor functional disorders, such as achalasia and gastroesophageal reflux disease, using high-resolution manometry and multichannel intraluminal impedance pH monitoring. We have started to use mesh to reinforce the hiatus in esophageal hiatal hernia repair. Because a correlation is strongly suspected between the viability of the gastric tube assessed with an intraoperative thermal imaging system during esophagectomy for esophageal cancer and the postoperative complication rate of a graft, such imaging has become standard practice. To reduce the rates of postoperative recurrent nerve palsy, we continue to monitor the activity of the recurrent nerve during surgery; the results will be analyzed and will be published soon.
To ensure the benefits of laparoscopic sentinel-node navigation surgery with an infrared endoscope for early gastric cancer, we are collecting data to compare the outcomes of conventional surgery and less invasive surgery. We have started to perform various types of immunohistochemical staining and to examine the expression of messenger RNA in tumor cells. We found that zinc finger protein 217 was an independent prognostic factor for relapse-free survival in patients with gastric cancer, which might be used as a

novel prognostic biomarker of 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, multiple postoperative gastrointestinal function tests are applied to patients who have undergone gastrectomy to evaluate various gastrectomy procedures and to inform the patients of the appropriate management.

Lower gastrointestinal surgery
We assessed surgeons' stress during laparoscopic surgery for colorectal cancer by measuring blood levels of adrenaline, noradrenaline, dopamine, ACTH, and cortisol. We are analyzing the data to determine which factors are related to surgeons' stress during an operation. We have published an article describing the excellent cosmetic outcome of a novel patient-friendly ileostomy procedure. This procedure uses the umbilical fossa for placement of a defunctioning ileostomy followed by a simple umbilicoplasty for ileostomy closure. A collaborative study with the Department of Urology to identify novel cancer-related proteins of the gastrointestinal tract is ongoing. The relationship between copy number variation and the recurrence/prognosis is evaluated after the extraction of DNA from frozen specimens of colorectal cancer tissue, because copy number variation may influence gene functions. In collaboration with the Department of Biochemistry, we are 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 of colorectal cancer. As a first step of this project, the following basic research will be started: analyses of cell-cycle regulation and dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase (DYRK) in relation to c-jun/c-myc phosphorylation. In addition to these analyses, we will strengthen the foundation of our future basic research by applying the complementary DNA library and by constructing clinical database.

Hepatobiliary and pancreatic surgery
The outlines of our main research activities are as follows:
1. Live donor liver transplantation (LDLT) and regenerative medicine
2. Treatment for hepatocellular carcinoma 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 with eltrombopag after splenectomy for idiopathic thrombocytopenic purpura
10. Molecularly targeted therapy for advanced hepatocellular carcinoma
11. Analyses of new biological tumor markers for hepatocellular carcinoma

The first LDLT was successfully performed for a patient with postnecrotic cirrhosis and hepatocellular carcinoma on February 9, 2007. Our 13th LDLT was performed for a patient with primary biliary cirrhosis on May 31, 2013. All 13 recipients were discharged in good condition on postoperative days 15 to 55, and all donors were discharged on postoperative day 8 to 26 and returned to their preoperative status. We are planning to extend the indications of LDLT to blood type-ABO incompatible transplants and to acute hepatic failure. In our department, the 5-year cumulative overall survival rate after hepatic resection for hepatocellular carcinoma is 70%, which is significantly better than the nationwide data of 52% in Japan.

We have performed translational research on combination chemotherapy with gemcitabine and a new protease inhibitor, nafamostat mesylate, which is associated with both the inhibition of nuclear factor κ-B and the induction of apoptosis in pancreatic cancer cell lines. We have started a new combination chemotherapy protocol with gemcitabine, TS-1, and nafamostat mesylate for advanced pancreatic cancer.

We have also performed extended liver resections as conversion therapy to multiple metastatic liver tumors, mainly originating from colorectal cancers. Otherwise, the indications for laparoscopic surgery, including hand-assisted laparoscopic surgery and laparoscopy-assisted, i.e., hybrid surgery, to hepatobiliary, pancreatic, and splenic diseases have been gradually expanded due to its lesser invasiveness. Navigation for liver resection has been covered 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 as well as experimental studies including 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 are ongoing. Also, we have started to apply molecularly targeted therapy to advanced hepatocellular carcinoma and to analyze new biological markers for hepatocellular carcinoma.

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Department of Surgery
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General Summary

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

Research Activities

Chest Surgery

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

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

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

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

Our clinical studies are also evaluating new devices and methods, such as narrow-band

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

Breast

1. Clinical study

1) The evaluation of sentinel lymph-node biopsy after neoadjuvant chemotherapy

The disease status of the axillary lymph nodes is the most important prognostic factor for breast cancer. However, axillary lymph-node dissection is associated with postoperative morbidities, such as upper extremity edema, pain, paresthesia, and restriction of the shoulder girdle. The minimally invasive technique of sentinel lymph-node biopsy produces less morbidity and yet allows accurate pathologic staging of the axilla. Experience with sentinel lymph-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 who have breast cancer treated with neoadjuvant chemotherapy.

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

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. With Sonazoid the sensitivity of ultrasonography for detecting small cancers of the breast equals that of magnetic resonance imaging.

3) Evaluation of psychiatric illnesses of patients with breast cancer

Many women experience emotional distress, depression, and anxiety after a diagnosis of breast cancer. We have prospectively analyzed patients with breast cancer who have undergone surgery.

4) Evaluation of the effectiveness of exercise for psychiatric illnesses in patients after surgery for breast cancer

We prospectively investigated the effects of exercise on psychological health

5) Evaluation of the beneficial effects of cryotherapy for small cancers of the breast (Kashiwa)

Minimally invasive cryoablation techniques are being included in research protocols to treat small cancers of the breast. These techniques offer the advantage of improved cosmesis.

2. Basic research

1) Studies of the early development of breast cancer

As screening mammography has become more common in Japan, the prevalence of ductal carcinoma in situ has increased to account for 20% of breast cancers in Japan. We have used immunohistochemical techniques to study biological factors involved in the progression of ductal carcinoma in situ to invasive breast cancer.

2) The evaluation of clinically useful biomarkers for triple-negative breast cancer

Triple-negative breast cancer (TNBC) tumors do not express estrogen receptor or progesterone receptor and do not overexpress human epidermal growth factor receptor 2. Historically, TNBC has responded well in the neoadjuvant setting, with rates of pathologic complete response commonly higher than for other types of breast tumor. However, more than half of patients with TNBC do not achieve a pathologic complete response and have an extremely poor prognosis. Gene-expression profiling has demonstrated that TNBC is a highly heterogeneous disease, including 2 basal-like, immunomodulatory, mesenchymal, mesenchymal stem-like, and luminal androgen receptor subtypes. By analyzing biological markers, we have attempted to identify chemosensitivity factors in TNBC.

3) The detection of circulating tumor cells in the bone marrow

The presence of circulating tumor cells in the peripheral blood and the bone marrow of patients with breast cancer is an independent prognostic factor. We are studying the prognostic value of the presence of circulating tumor cells in the bone marrow of patients receiving chemotherapy.

Endocrine

1. Basic research

1) The detection of antigens of thyroid carcinoma in serum or urine

A monoclonal antibody, designated JT-95, was made against a thyroid papillary carcinoma obtained by our Department of Breast and Endocrine Surgery. We are attempting to measure the antigen recognized by JT-95 in the serum or urine of patients with papillary carcinoma, in collaboration with the Division of Molecular Cell Biology of The Jikei University. The quantity of antigen of JT-95 is higher in patients with papillary carcinoma, especially those with metastasis to lung or bone, than in patients with breast carcinoma.

2) Research regarding the metastasis of thyroid carcinoma to the lymph nodes

Thyroid papillary carcinoma tends to metastasize to lymph nodes. On the other hand, follicular carcinoma tends to metastasize hematogenously to the lungs and bone. We co-cultured a papillary carcinoma cell line (SW1736) and a lymphoma cell line (Daudi) with or without the JT-95 antibody to examine changes in cell attachment. We found that the adhesion between cells was inhibited in proportion to the quantity of JT-95 added. We are investigating the mechanism of cell-to-cell inhibition.

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Department of Surgery
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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 drug-eluting stents, and clinical studies of specific antibodies for heparin-platelet factor 4 (PF4) complexes.

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-Closure™, 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 studies

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.

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 thorough one side of a carotid artery, after which a covered stent is inserted as a branch and deployed into the stent graft (in an in-situ retrograde fashion). We have examined this retrograde in-situ branched surgery in an in-vitro study and have applied it clinically. This operation is expected to be a less invasive surgery for aortic arch aneurysms. We also use branched thoracic arch stent grafts 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 stents in the superficial femoral artery

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

Both a global registry and a randomized controlled trial, in which most patients were enrolled in the United States, but also in Germany and Japan. We participated in this trial. After 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 antibodies against heparin-PF4 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-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

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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 fracture risk. High levels of pentosidine in urine or blood and mild hyperhomocysteinemia, which suggest bone collagen abnormalities, might be used as surrogate markers for evaluating bone quality and for assessing the risk of bone fracture. 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 repairs of bone defects. Furthermore, studies of the relationship between micropores and osteogenic factors, such as bone morphogenic proteins (BMPs), have facilitated further understanding of the mechanism of osteogenesis.

Clinical Research

Our clinical practice has been divided into 9 subspecialties to treat a wide range of musculoskeletal disorders and is managed by different specialist teams: shoulder joint, hand surgery, spine, hip joint, knee joint, foot surgery, trauma, osteoporosis, and rheumatic diseases. All teams maintain a high level of expertise and are actively involved in scientific activities. The spine team has demonstrated the effectiveness of minimally invasive spine stabilization with the S2-alar-iliac screw for elderly patients with spinal deformities, especially sagittal imbalance in the lumbosacral region. The spine team has investigated surgical outcomes in patients treated with this technique. The knee joint team has been performing total knee arthroplasties with patient-matched instrumentation and has analyzed the effectiveness of cutting-edge technologies even more advanced than the surgical navigation system itself. Through this range of clinical research activities, all teams fulfill their important roles at a clinical academic hospital, and their commitment has been highly evaluated.

Research Activities

Outcomes of surgical treatment for proximal humerus fractures using multiaxial fixator plates

The outcomes of surgical treatment using multiaxial fixator plates for proximal humerus fractures in 9 patients were reviewed. The patients' mean age at surgery was 67 years,

and the average follow-up period was 2 years 9 months. Bone union had developed in all patients within 4 months. Avascular necrosis was not found in any patient after surgery. The mean ranges of motion at the final follow-up examination were 123-degrees elevation and 49-degrees external rotation. The mean Japanese Orthopaedic Association score was 86 points. The surgical treatment for proximal humerus fractures using multiaxial fixator plates produced satisfactory results. This system is indicated for markedly displaced 2- or 3-part fractures and even for 4-part fractures in young patients.

The current status of hand surgery

We treat many kinds of disease, from trauma, such as fractures, tendon ruptures and neurovascular injuries, to degenerative diseases and tumors. We also provide special surgical techniques for suturing tendons and for microsurgery. Over the last 10 years we have performed 300 to 400 operations of various types per year. After surgery, we cooperate with occupational therapists in the outpatient clinic to help patients achieve functional recovery. In clinical research, we analyzed collagen cross-linking in the hands of patients with Dupuytren contracture to clarify the etiology of this disease. We have started to administer antibodies against RANKL (receptor activator of nuclear factor kappa B ligand) to patients with recurrent or unresectable giant cell tumors of bone.

Surgical treatment for elderly patients with spinal deformities with a focus on sagittal imbalance at the lumbosacral region

Spinal imbalance negatively affects the quality of activities of daily life, especially in elderly people; therefore, surgery is occasionally performed to correct the spinopelvic alignment and to restore good sagittal balance. Although surgical treatments for elderly patients are invasive in terms of blood loss and operation time, we were able to reduce the effects of these factors by using a lateral access approach for lumbar spine fixation and to obtain good coronal or sagittal alignment. However, other factors that influence clinical outcomes include rigidity of the deformity and comorbidities, such as osteoporosis. Thus, strong fixation systems that provide efficient functional support of the lower lumbosacral vertebrae are extremely important. The S2-alar-iliac screw is one of strongest anchors at the lowest vertebral segments and is useful for spinopelvic re-alignments. We believe that the combination of the lateral access approach for lumbar spine fixation and instrumentation with the S2-alar-iliac screw system is extremely beneficial for treating elderly patients with spinal malalignment. Surgical outcomes of this procedure are being evaluated.

Treatment of infected total hip arthroplasty with a 3-stage articulating cement spacer method and preservation of the biologically fixed cementless stem

We have tried to preserve a tightly fixed stem in cases of cementless total hip arthroplasty (THA) and to create an articulating antibiotic-loaded cement spacer after removal of the acetabular cup with the twin aims of controlling infections and preserving hip function. Six cases of chronic deep infection after cementless THA were studied. Infections developed after 2 bipolar hip arthroplasties (BHAs), 2 primary THAs, and 2 revision THAs. The infections were successfully controlled in all 6 patients, and the second-

stage reconstructions were performed. Biological fixation of the cementless stem complicates implant removal while acting as barrier against bacterial invasion. An articulating acetabular cement spacer combined with a preserved cementless stem provided both infection control and preservation of hip function in all 6 patients.

Patient-matched instrumentation method in total knee arthroplasty: a prospective study of the accuracy of different patient-specific bone-cutting guides
Preoperative and intraoperative patient-specific templating has gained attention as the next technological development after computer-assisted surgery navigation systems in knee surgery. In our department, we have been evaluating the accuracy of implant positioning during total knee arthroplasty with patient-matched instruments and carrying out a comparative study against the computer-assisted navigation system. The evaluation also includes a comparative trial against conventional surgery, analysis of 3-dimensional reconstructions, and development of more-precise preoperative planning software. The comparative analysis of the accuracy of different patient-matched instruments is being carried out in a prospective manner.

Artificial reproduction of the weight-bearing state for the foot and ankle using a loading device designed for use with conventional computed tomography scanners
Computed tomography images obtained in the standing position are highly desirable for evaluating 3-dimensional bone alignment in foot disorders. We have designed a prototype loading device that can be used with a conventional computed tomography scanner and evaluated the reproducibility of the obtained data by comparing them with variables obtained in the standing position. The same axial pressure load corresponding to body weight was applied in 5 healthy volunteers lying in the supine position. With the subject's foot on the prototype device, the sole-ground contact area and the maximum-sole and center-pressure positions were measured and were compared with those obtained in the standing position. We could not find any significant differences between any of the respective values. Our prototype device allowed approximation of plantar load distribution and strength to those measured in the standing position.

Estimating bone material quality in the context of bone and vascular linkage
A reduction in sex hormone levels from middle age onwards, increasing age, and an overall increase in oxidative stress due to lifestyle-related diseases can reduce the quality of bone material in terms of collagen posttranslational modifications and cross-link formation. The intermolecular cross-link formation of collagen, which regulates bone-material properties, is a mechanism independent of bone remodeling. In other words, cross-link formation is controlled by the environment surrounding the bone matrix and is, therefore, influenced by cellular functions, oxidative stress, and glycation processes that occur in this environment. Because oxidative stress is also a risk factor for arteriosclerosis and cardiovascular events, low bone quality and arteriosclerosis should also be linked. High levels of pentosidine in urine or blood and mild hyperhomocysteinemia, which indicate abnormalities of bone collagen, might be used as surrogate markers for evaluating bone quality and for assessing the risk of bone fracture.

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General Summary

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

Research Activities

Cerebrovascular diseases・Endovascular surgeries

1. Analysis of the natural history of unruptured intracranial aneurysms

Since 2003, more than 4,000 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 in human intracranial aneurysms using computational fluid dynamics

Owing to the research 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 imaging software for analyzing 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, and the quality of the

images is being analyzed. By significantly reducing artifacts due to metal coils, this software has significantly improved visualization near the coil mass in C-arm CT images. The software has recently become commercially available for angiography devices built by Siemens Medical Systems (Erlangen, Germany). 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.

4. Development of a novel intracranial stent device for treating 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 (2.1 Fr system compatible) 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.

5. Development of a novel bioactive coil device for treating 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 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 users. 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 using an online bulletin board system. The application has been released in collaboration with NTT Docomo, which is the Japan's largest mobile service provider, with more than 60 million customers.

Brain tumor

The current therapeutic standard for malignant glioma includes surgery followed by concomitant radiation and chemotherapy with temozolomide. The median survival time of patients with glioblastoma multiforme, the glioma with the highest grade of malignancy, is 15 months, despite aggressive treatment with surgical resection, radiotherapy, and temozolomide chemotherapy. Although the cytotoxic effect of temozolomide correlates with epigenetic silencing of the *MGMT* gene by promoter methylation in tumor cells, there are few therapeutic options for patients with temozolomide-resistant glioma. A novel therapy to improve the prognosis of patients with temozolomide-resistant glioma is, therefore, eagerly awaited, and "chemoimmunotherapy," a synergism of chemotherapy and immunotherapy, is one such novel paradigm that has been investigated for different types of tumors, including 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 TAA-derived 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. As the efficient isolation and preparation of human and murine DCs have become possible, the strategies to elicit effective uptake of TAAs by DCs have gained special interest for developing novel cancer immunotherapies. We have previously shown in a phase 1 clinical trial that immunotherapy for glioma with fusions of DCs and glioma cells induces safe, tumor-specific immune responses. The therapeutic significance of recombinant interleukin (IL)-2 supplementation in fusion cell (FC) immunotherapy has also been shown in a phase 2 clinical trial. A strategy to induce high levels of IL-12 production by FCs might thus enhance the antitumor responses in FC immunotherapy. In another study, we observed that polyinosinic: polycytidylic acid (Poly[I:C]) transfection induced high levels of 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 31st 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. FC immunotherapy has been submitted to the Ministry of Health, Labour and Welfare for approval as an advanced medical treatment.

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, The Jikei University Hospital Women's & Children's Medical Center was established in October 2002. In the last 10 years we have treated more than 1,500 new cases of various entities, including, spina bifida, hydrocephalus, craniofacial anomalies, and brain tumors. Now our division has been run by three staffs since 2012.
In the field of hydrocephalus research, extensive pathophysiological analyses of CSF dynamics have been performed in both the fetal and postnatal periods. On the basis of these large series of clinical cases with extensive clinical investigations, we have proposed a unique theory for the specificity of CSF dynamics in the immature brain. We have also completed the development of a new neuroendoscope and have proposed a new surgical technique with or without the use of a neuronavigation system.
We have been collecting the largest series of patients with spina bifida. We have been promoting national and international cooperative studies on controversial issues in this field.
In the field of craniofacial anomaly research, we have extensively applied the distraction method to Japan's largest series of cases; the clinical efficacy of this method has been summarized, and our extensive work has received the honorable prize of the International Society for Pediatric Neurosurgery, Raimondi's Award in 2004, and the Kawabuchi Award in 2005.
Our clinical and research activities have also been well maintained on the basis of firm international collaboration with world-leading pediatric neurosurgeons and related researchers.

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Department of Plastic and Reconstructive Surgery

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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

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 years after complete digital separation. Evaluation included the range of motion in the hands, radiographic examination, and the Disabilities of the Arm, Shoulder, and Hand Questionnaire score. According to the 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 follow up long term underwent mental development testing, which showed IQs 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 the 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 more evaluation is needed to understand the long-term affects of Apert hand on patients, especially those of normal intellectual ability.

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 nasomanometory 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 columella strut and 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.

Introducing the techniques of aesthetic surgery in open septorhinoplasty

Rhinoplasty has 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.

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 pharyn-

golaryngectomy or hypopharyngectomy with laryngeal preservation. The defects were classified into 4 types on the basis of the area of the hypopharyngeal defect. Functional results were evaluated by means of routine physical examination, variables related to swallowing, and X-ray barium deglutition examination. Perioperative mortality and morbidity were reviewed. There were no perioperative deaths, and 98% of the flaps survived. Forty-three patients (80%) were able to eat an unrestricted diet and experienced no aspiration. 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 the resection of head and neck cancers was examined. A total of 188 patients who underwent head and neck reconstruction after cancer extirpation from January 2010 through December 2011 were studied. The predicted risk of complications was calculated with the POSSUM and compared with the actual rate of perioperative complications. Perioperative complications occurred in 35 patients (19%) and consisted of systemic complications in 17 patients (9%) and surgical site infection in 20 patients (11%). The patients were divided into a perioperative complication group and a no-complication 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

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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 a new field — transcatheter aortic valve replacement — and we started preparing to perform such operations. We are also preparing to establish a left ventricular (LV) assist device program. 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. The major activities are described below.

Research Activities

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

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

We investigated the impact 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.

Clinical studies of myocardial protection during pediatric heart surgery

In infants with ventricular septal defect, atrioventricular septal defect, or other congenital malformations who underwent open heart surgery with various cardioplegic strategies, biochemical markers for myocardial injury (troponin T) and oxidative stress (8-iso-prostane) were measured intraoperatively. This retrospective study confirmed the benefits of BCP with terminal warm BCP reperfusion (hot shot) over crystalloid cardioplegia on reperfusion-induced biochemical injury.

Clinical studies of surgical outcomes of patients with univentricular heart

1. 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 (BDG) 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 pulmonary artery (PA) pressure (PAP) $\geq$ 15 mm Hg or peripheral vascular resistance (PVR) index $\geq$ 3.0 Wood units for the pulmonary factor and systemic ventricular end-diastolic pressure (EDP) $\geq$ 12 mm Hg for the ventricular factor. Multivariate regression analysis revealed that the presence of additional PA flow and the age when the BDG 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 BDG procedure before 12 months and 8 months respectively, than in older patients. In conclusion, the optimal timing of the BDG procedure is younger than 12 months for pulmonary risk factors and younger than 8 months for ventricular factors.

2. Optimal interval between the BDG procedure and the final Fontan procedure and the role of pulmonary vasodilator therapy

To evaluate the effects of the interval after the BDG procedure on the Fontan Risk Profile and the role of pulmonary vasodilator therapy, we analyzed changes in the Fontan Risk Profile (pulmonary or ventricular variables) during the interval between the BDG procedure and the Fontan procedure in 20 high-risk patients with complicated issues who underwent catheterization 2 or more times after the BDG procedure.

There was no significant change in PAP, PVR, EDP, or the PA index up to 12 to 24

months after BDG. However in high-risk patients who had been treated with bosentan or sildenafil, PAP and PVR decreased significantly 6 months after the BDG procedure. From these findings we conclude that: 1) during the interval after BDG of 3 to 24 months, PA index remained unchanged without progressive decreases, and 2) in high-risk patients with elevated PAP, pulmonary resistance, and EDP, a prolonged observation interval before the Fontan procedure associated with medication therapy (pulmonary vasodilator or angiotensin-converting enzyme inhibitor)has beneficial effects on risk 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 mm^2/m^2 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 pressure (CVP) and lower cardiac index, 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.

4. Clinical study of the usefulness of the intraoperative Fontan simulation test under BDG circulation to predict Fontan outcome

As a strategy for high-risk surgical candidates, we proposed a novel method of intraoperative Fontan simulation testing under BDG circulation and applied this method to 20 high-risk Fontan candidates. Before CPB, the pulmonary flow-pressure (PAP and left atrial pressure [LAP]) relationship was analyzed by stepwise volume loading from a cannula, up to a preoperative pulmonary flow index of 2.5 $L/min/m^2$ body surface area, where effective PA flow is measured with a transit flow meter placed around the superior vena cava, with concomitant measurement of PAP and LAP. With the assumption that the minimum requirement of the cardiac index for successful Fontan during the acute phase is 2.5 $L/min/m^2$, predicted CVP at Fontan completion was calculated as transpulmonary pressure gradient at a PA flow index of 2.5 plus baseline LAP before volume loading. We have shown a significant relationship between predicted CVP and CVP on the first postoperative day, suggesting a role for this index as a predictor of acute hemodynamic status.

Clinical study of adult cardiac surgery

1. Mitral valve repair in active infective endocarditis

For patients with mitral valve mitral valve infective endocarditis (IE), mitral valve repair is preferred over mitral valve replacement. The objective of this study was to investigate the limitations of mitral valve repair for treating active IE by reviewing our recent operations for active IE. From January 2004 through August 2012, 24 patients with active IE underwent mitral valve surgery. The mean age of patients was 60 ± 16 years. The active IE was classified into 4 types according to the severity of mitral valve destruction, and surgical treatments were selected for each: type I, vegetation with less destruction of leaflets and subvalvular apparatus, debridement by rubbing and ring annuloplasty; type II, 1 localized lesion, resection and suture; type III, 2 or more destructive lesions, resection and suture or patch augmentation with artificial chordae; and type IV, destruction extending over annular lesion, reconstruction of annuli with pericardial patch. We assessed the surgical outcomes of these patients with a focus on the validity of mitral valve repair for active IE. Of the 24 patients, 21 (87.5%) underwent mitral valve repair and 3 underwent mitral valve replacement. The surgical outcomes for both procedures were satisfactory. There were no late deaths and no recurrence of IE. The patients who underwent mitral valve replacement had type III IE (2 patients) or type IV IE (1 patient) and were the first in this cohort to be treated. In conclusion, mitral valve repair for active IE is a useful treatment for most cases and can be achieved with radical resection of infected portions and coverage of defects with a pericardial patch supported by artificial chordae. In cases of wide invasion or destruction, which should be resected, more complex procedures are needed. Therefore, early surgical intervention should be considered for successful mitral valve repair and for higher survival rates.

2. Use of mechanical aortic valves in patients older than 65 years

The first choice of aortic prosthesis in patients older than 65 years is a biological valve. However, new mechanical valves, which have a small annulus and larger effective orifice, have been implanted in elderly patients, but morbidity can be significant if the annulus is not enlarged. An increasing number of patients older than 60 years are receiving mechanical valves. The main reason mechanical valves are chosen, even for elderly patients, is chronic atrial fibrillation, which can be refractory to treatment and necessitate anticoagulation with warfarin. In this study, the validity of mechanical valve selection for patients older than 65 years was examined. Of patients aged 65 to 70 years, 32% strongly desired to receive a mechanical valve because of recent increases in mean life span. The 10% of patients older than 75 years elected mechanical valves because of the low invasiveness of their implantation when the calcified small annulus and ascending aorta are not enlarged. The other patients with structural valve deterioration (SVD) of a previous biological prostheses desired mechanical valves so that reoperation could be avoided. Our studies suggest: 1) the frequency of choosing mechanical valves in the young-old population has increased, 2) because the likelihood of postoperative treatment with warfarin is high, we have selected mechanical valves for patients with long-lasting atrial fibrillation undergoing multiple valve surgeries, and 3) we have implanted new small mechanical valves without annular enlargement in patients older than 80 years (most of whom are female) with a small annulus. However, the frequency of bleeding complications caused by treatment with warfarin has been low, and the mid-term results have been good. The validity of using mechanical valves for elderly patients should be

examined after long-term follow-up.

3. The role of a perioperative heart team composed of intensive care unit physicians and rehabilitation staff in dealing with the hemodialysis patients who underwent coronary artery bypass grafting

Recently, the number of patients receiving hemodialysis (HD) who undergo coronary artery bypass grafting has increased. In these patients, the risks of both surgical complications and morbidity is high. At our institution, a cooperative team has contributed to the recent excellent operative outcomes of these patients. The heart team decides on perioperative care after discussing treatment plans every morning. Because perioperative care takes place in the intensive care unit (ICU), the burden on the general wards has been relieved. We have introduced preoperative dental care, HD management during early admission, off-pump coronary artery bypass surgery, preoperative administration of amiodarone, the avoidance of using bilateral internal thoracic arteries, the control of blood glucose to less than 120 mg/ml, and the appropriate timing for the start of postoperative HD. After the transition from continuous to intermittent HD has been confirmed, patients are moved to the general wards. The ICU has 20 beds and is staffed by 8 ICU specialists. There are also 2 clinical engineers, 3 pharmacists, 60 nurses, and periodic rounds by the infection control team and dentists. Postoperative rehabilitation is started as soon as possible in the ICU. To reduce the burden on the general wards, in the ICU ventilatory support is provided via endotracheal intubation, and adrenergic agents are continuously infused. Care in the ICU and the cooperation of other co-medical staff have contributed greatly to the excellent postoperative outcomes, regardless of the complexities and severities of recent patient diseases.

4. The economic pitfalls of valvular operations for patients aged 80 years or older

Operations for patients aged 80 years or older have become more common because of the recent growth of this segment of the population and the progression of medical technologies. Because an understanding of current economic problems of aged patients is important for discussing recent medical politics, we examined aged patients' medical charges of valvular operations. For aged patients, the cost of the diagnosis procedure combination (DPC) was higher than for adults in general, and their postoperative hospital stays were significantly longer. Perioperative care for aged patients are generally considered to be an excess burden for medical workers. On the contrary, the income of DPC showed no significant difference between young and old patients. So some additional fees must be needed for lower current estimation of the care of aged patients.

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Department of Obstetrics and Gynecology

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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. Profiling of actionable gene alterations in ovarian cancer by targeted deep sequencing

To construct a profile of therapeutically actionable gene alterations in the major histological types of ovarian cancer. Seventy-two Japanese patients with surgically resected ovarian cancers were selected from an original cohort consisting of 267 patients who had not received pre-treatment before surgery. Somatic mutations and copy number alterations at 740 hot spots in 46 cancer-related genes were detected by deep sequencing genomic DNA using a next generation sequencer. *TP53*, *PIK3CA*, and *KRAS* were the three genes with the highest frequency of mutations and were altered in 28 (38.9%), 18 (25.0%), and 10 (13.9%) ovarian cancer patients, respectively. Actionable mutations and/or copy number aberrations in nine other genes, *PIK3CA*, *KRAS*, *PTEN*, *ERBB2*, *RB1*, *CDKN2A*, *AKT1*, *CTNNB1*, and *NRAS*, were detected in 35 (48.6%) patients with ovarian cancer. These mutations tended to occur in a mutually exclusive manner. Non-serous histological type tumors showed frequent actionable gene alterations (32/47; 68.1%). The profile indicates that in the non-serous ovarian cancers found in this Japanese population there are frequent gene aberrations that link to therapy using molecular targeting drugs.

2. Promising Therapeutic Target of IL-6/IL-6R Signaling Pathway in Ovarian Clear Cell Carcinoma

Cytokine expression in a tumor microenvironment can impact both host defense against the tumor and tumor cell survival. We previously reported that ovarian clear cell carcinoma (CCC) showed a dominant Th-2 cytokine expression pattern driven largely by *IL-6* expression. The unique cytokine expression pattern found in CCC may be involved in the pathogenesis of this subtype. Modulation of IL-6 expression or its related signaling pathway may be a promising strategy of treatment for CCC.

3. MicroRNA-21 is a Candidate Driver Gene for 17q23-1 25 Amplification in Ovarian Clear Cell Carcinoma

Ovarian clear cell carcinoma (CCC) has unique clinical characteristics and behaviors that differ from other histological types of epithelial ovarian carcinoma (EOC), including a frequent association with endometriosis and a highly chemoresistant nature, resulting in poor prognosis. However, factors underlying its malignant behavior are still poorly understood. The aim of this study was to investigate the role of *miR-21* in 17q23-25 amplification associated with CCC oncogenesis. We identified 17q23-25 copy number aberrations among 28 primary CCC tumors by using a comparative genomic hybridization method. Next, we measured expression levels of the candidate target genes, *miR-21* and *PPM1D*, for 17q23-25 amplification by real-time RT-PCR analysis and compared those data with copy number status and clinicopathological features. In addition, immunohistochemical analysis of PTEN (a potential target of *miR-21*) was performed using the same primary CCC cases. We investigated the biological significance of *miR-21* overexpression in CCC using a loss-of-function antisense approach. 17q23-25 amplification with both *miR-21* overexpression and PTEN protein loss was detected in 4/28 CCC cases (14.2 %). The patients with 17q23-25 amplification had significantly shorter progression-free and overall survival than those without 17q23-25 amplification (log-rank test: $p = 0.0496$; $p = 0.0469$, respectively). A significant correlation was observed between *miR-21* overexpression and endometriosis. Both *PTEN* mRNA and PTEN protein expression were increased by *miR-21* knockdown in CCC cells. We also confirmed that *miR-21* directly bound to the 3′-untranslated region of *PTEN* mRNA using a dual-luciferase reporter assay. *MiR-21* is a possible driver gene other than *PPM1D* 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* tumor suppressor gene.

4. Evaluation of maintenance docetaxel after paclitaxel and carboplatin combination chemotherapy in ovarian cancer

To test the concept of taxane sequencing, this feasibility trial evaluated maintenance docetaxel after paclitaxel and carboplatin combination chemotherapy in patients with stage Ic-IV ovarian cancer. All patients received debulking surgery followed by paclitaxel and carboplatin chemotherapy. Maintenance docetaxel started at an initial dose of 70 mg/m^2 every 4 weeks for 6 cycles and was extended to 10 cycles when effective. Stage subsets in 20 eligible patients were as follows: Ic, 1 patient (5 %), II, 1 patient (5 %), III, 13 patients (65 %), and IV, 5 patients (25 %). Neutropenia was common (40 % with grade 3 or 4) and was most frequent during first or second cycle although the disabling peripheral neuropathy was not observed. Twelve patients completed protocol therapy (6≦cycles), while 8 patients failed to complete 6-course chemotherapy, because of progressive disease (5 patients) or grade 4 toxicities (3 patients). Median overall survival from the start of maintenance chemotherapy was 39 months with median follow up period of 40 months and 1-year survival rate was 100 %. Those data were translated into the following conclusion; no less than 6 cycles of single-agent docetaxel maintenance chemotherapy is feasible and generally tolerable to women with advanced ovarian cancer who attained a clinically defined response to initial paclitaxel and carboplatin

based chemotherapy.

5. Feasibility Study of the Laparoscopic Approach for Borderline Ovarian Tumors

Compared to ovarian cancers, borderline ovarian tumors (BOTs) primarily present at an early stage in younger patients and have an excellent overall prognosis. Clinical management of BOTs during reproductive age has been modified from radical surgery to fertility-sparing surgery. However, the accurate diagnosis of BOTs prior to surgery is currently difficult. The aim of this study was to evaluate the feasibility of the laparoscopic approach for BOTs in terms of clinical outcome, including pre and intra-operative diagnosis. From January 2005 through December 2012, we retrospectively reviewed the clinical and surgical parameters of patients undergoing surgery for epithelial BOTs at our institution. A total of 119 BOTs were analyzed. For initial surgery, 111 (93%) underwent a laparotomy, and 8 (7%) underwent laparoscopic surgery, All the cases that underwent laparoscopic surgery were selected under a preoperative diagnosis of adenoma. Among 119 BOTs, 70 (64%) had solid areas and 50 (82%) had contrast enhancements in the tumor that was revealed by magnetic resonance imaging. The accuracy of intraoperative frozen section diagnosis was 84%. The incidence of tumor rupture during surgery was significantly higher in the laparoscopic surgery group compared to laparotomy group ($P = 0.0007$); however, there was no significant difference in the recurrence rate between stage Ia and Ic (b) patients. Pre- and intra-operative characterization of ovarian tumors using enhanced imaging studies and frozen section is clinically important. Although further studies are needed, with appropriate patient selection, laparoscopic surgery might be an acceptable intervention for young women with BOTs.

Perinatology

1. Multiple injections of anti-mouse β2-glycoprotein 1 antibody induce FcR gamma-dependent fetal growth restriction in mice

Antiphospholipid syndrome (APS) is characterized by the presence of circulating antiphospholipid antibodies (aPLs). It is also a leading cause of thromboembolic events, repeated miscarriages, and fetal loss and is a major risk factor for fetal growth restriction (FGR) and pre-eclampsia. Anti-β2 glycoprotein I (aβ2GPI) antibody is a human aPL that is considered a specific and important marker for APS. We developed a murine model of FGR by administering multiple injections of WBCAL-1, a well-characterized mouse aβ2GPI monoclonal antibody. Administration of WBCAL-1, but not of the control antibody of the same isotype and saline, into pregnant mice decreased the size of fetuses and placentas without affecting the number of delivered pups. Also, a significant increase in urinary albumin and electron microscopic changes, such as splitting layers of basal membranes in the placental labyrinth and rearrangement of pores in glomerular endothelial cells, were observed in WBCAL-1-treated mice. However, injection of WBCAL-1 did not induce any changes in blood pressure or in typical indicators of blood thromboembolic symptoms. Furthermore, our present findings suggest that proteinuria is a symptom associated with APS-related FGR with placental and renal tissue injuries and that FcR-gamma is a molecular target for preventing aß2GPI antibody-mediated obstetric pathologies.

2. Cytotrophoblast alterations in placentas from disorders associated with aPLs and dysregulated clotting factors

Both aPLs and clotting factor disorders have been recognized as causes of placental insufficiency and obstetrical complications. Approximately 30% of patients have aPLs (14.5%) or clotting factor disorders (12.7%). To investigate the effect of a combined aspirin and heparin therapy on next-pregnancy outcome, we compared pregnancy outcomes and placental pathologies in 2 successive pregnancies in the same patients with aPLs or clotting factor disorders. Therapies in the second pregnancy were effective in terms of delivery week for all cases, and in terms of fetal weight, which increased significantly in patients with aPLs compared with those in patients with clotting factor disorders. The fibrin regions in the extravillous areas of the placenta increased significantly in patients with clotting factor disorders but not in patients with aPLs. Hence, there is a need to further assess the effects of aPLs and clotting factor disorders on placental development.

3. Immunohistochemical localization of bilirubin oxidation in human placenta

Pregnancy is a state of oxidative stress, and in certain pathologic pregnancies the stress has been recognized to be heightened. However, the response in human placenta has not been fully clarified. 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. Then in this study, to elucidate whether the oxidative stress affects placental physiology and function, this heme catalytic pathway and oxidative response in human placenta were morphologically investigated. 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). Immunoreactivity with EP139Y was demonstrated around decidual spiral arteries, especially those with atherosis, in areas of infarction and villous stroma accompanied by perivillous fibrin deposits, and in syncytial knots. Immunoreactivity for 24G7 was identified around areas of infarction and decidual vasculopathy. Staining for bilirubin and HO-1 was more diffuse and more intense in cases of pre-eclampsia than in uncomplicated cases. This is the first report to demonstrate the localization of HO-1 and biopyrrins immunohistochemically in the human placenta.

Reproductive endocrinology

In 2013 we researched infertility counseling, endoscopic surgery, fertility preservation undergoing suffer from gonadotoxic agents, and women's sport medicine.

We assessed how the information is provided for infertility treatment. The desire for counseling is low among persons who do not have information about the treatment.Infertility counseling about the "aging of the egg" and the "end of treatment" had increased. We have also examined the relationship between body-mass index and endometriosis for the early detection of this condition. We have examined fertility preservation with respect to the growing need for it in our hospital. The need for oral conceptive pills by female athletes to treat dysmenorrhea was considered. Numerous cases of recurrent pregnancy loss in clinical trials were also followed.

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Department of Urology

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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; lithiases; technology and instruments, such as laparoscopy; transplantation; and female urology.

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) TMPRSS2: ERG fusion in Japanese patients with prostate cancer
2) Establishment and biological analysis of our new prostate cancer model, named JDCaP, derived from a Japanese patient
3) 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
4) Reduced expression of stem cell marker CD44v9 in urothelial basal cells in patients with interstitial cystitis/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) Clinical study of high dose rate brachytherapy with external beam radiation therapy for high-risk prostate cancer
2) Study of deep venous thrombosis after urological surgery
3) Study of the incidence of latent prostate cancer
4) Clinical study of nomograms for predicting unilateral pathological T3 prostate cancer
5) Study of cryoablation therapy for patients with small renal tumors
6) Urothelial dysfunction in patients with interstitial cystitis/bladder pain syndrome

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Department of Ophthalmology

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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 IOLs, and yellow IOLs. We implant these new IOLs and evaluate subsequent visual function.

Neuro-ophthalmology
1. We examined the relationship between nonarteritic anterior ischemic optic neuropathy (NAION) and genetic polymorphisms of enzymes influencing endothelial function. The genotype distribution of the G/T (Lys198Asn) polymorphism of the endothelin-1 (ET-1) gene varied significantly in patients with NAION. In persons with the TT genotype of the Lys198Asn polymorphism, NAION was more likely to develop than in persons with the GG genotype. We found an increased prevalence of a G/T polymorphism of the ET-1 gene in patients with NAION. Our data suggest that this polymorphism may be an important risk factor for NAION in the Japanese population.
2. A rare manifestation of a limited form of neuromyelitis optica (NMO) with a lesion of the trochlear nerve nucleus was demonstrated. Detection of anti-aquaporin 4 (AQP4) antibodies in patients with myelitis facilitates the diagnosis of a limited form of NMO in

cases without optic neuritis. The brain lesions of NMO predominantly involve the hypothalamus and areas of the brainstem surrounding the third and fourth ventricles. Ocular motor nerve palsy due to lesions of brainstem nuclei may manifest in NMO, although ophthalmoplegia is rarely reported.
3. The aim of the present study was to clarify the association of genetic variation of the AQP4 gene with susceptibility to anti-AQP4 antibody-positive NMO in a Japanese population. We found that an AQP4 promoter polymorphism, rs2075575, is significantly associated with an increased risk of anti-AQP4 antibody-positive NMO in our Japanese population. Our data suggest that the AQP4 polymorphism is an important risk factor for the development of NMO.
4. We reported a case of asymmetric papilledema with normal cerebrospinal fluid pressure and a case of acute disseminated encephalomyelitis presenting as papilledema followed by optic neuritis.
5. We reviewed traumatic optic neuropathy and ischemic optic neuropathy and lectured on the diagnosis and treatment of neuro-ophthalmologic disorders.

Ocular oncology and histopathology
1. Immunoglobulin (Ig) G4-related ophthalmic disease belongs to a category of ocular adnexal lymphoproliferative disorders, the most frequent group of orbital tumors and simulating lesions. The aim of this study was to clarify the number of IgG4-related diseases among orbital lymphoproliferative disorders and to correlate the age and sex of such patients from 18 centers in Japan. A total of 1014 patients with orbital lymphoproliferative disorders were enrolled in this study. All had lymphoproliferative disorders pathologically diagnosed with surgical samples of ocular adnexal tissue. Of the 1014 patients with orbital lymphoproliferative disorders, 404 (39.8%) had extranodal mucosa-associated lymphoid tissue (MALT) lymphoma, 156 (15.4%) had other malignant lymphomas, 191 (18.8%) had non-IgG4 orbital inflammation, 219 (21.6%) had IgG4-related orbital inflammation, and 44 (4.3%) had IgG4-positive MALT lymphoma. The median age of patients with IgG4-related orbital inflammation was 62 years and was significantly lower than that of patients with MALT lymphoma (66 years) and higher than that of patients with non-IgG4 orbital inflammation (57 years). The male:female ratio was 105:114 among patients with IgG4-related orbital inflammation. Nearly a quarter of orbital lymphoproliferative disorders in Japan are related to IgG4.
2. We reviewed the frequency of orbital tumors and pointed out some limitations of surveys of orbital tumors in Japan.

Glaucoma
1. Analysis with the Markov model of the Eeffects analysis of the a glaucoma examination program using the Markov model.
The Gglaucoma produces an irreversible visual field loss field of vision obstacle by the cause disease of the main visual impairment of this country, and it is said that Eearly detection and, treatment is are important until the progress period because subjective symptoms are poor. We used the Markov model to perform analyze the effects of analysis using the Markov model screening of adults for glaucomanow when glaucoma was

screened in adult eyes examination. The early detection and, early treatment in of the glaucoma is are also useful for 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 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 mmHg 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 normal-tension 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 mmHg and 13.7 ± 4.5 mmHg, respectively, and the The difference between 10-minute supine IOP and sit-

ting IOPthem (ΔIOP 10 min) was 3.43 ± 1.8 mmHg ($p < 0.05$). Sitting IOP and ΔIOP 10 min were significantly correlated ($r = 0.66$, $p < 0.0001$). The lower the sitting IOP was, the lower ΔIOP 10 min was.

Functional neuro-imaging

Diffusion tensor imaging is a noninvasive technique to visualize axonal construction. The optic chiasm cannot be visualized with conventional diffusion imaging caused by magnetic susceptibility artifact around the sphenoid sinus. Two novel methods were used to visualize crossed and uncrossed fibers of the optic chiasm with the diffusion tensor imaging technique. With the readout segmentation of long variable echo-trains (RESOLVE) technique, susceptibility artifacts can be reduced through a different method of scanning raw data. TOPUP, a part of brain analysis software distributed by University of Oxford, can also solve susceptibility artifacts by calculating the "real image" through paired distorted images with known differences in direction. These 2 methods could distinguish crossed and uncrossed fibers in the optic chiasm.

Developmental functional abnormality

Binocular summation on the visual cortex was explored with fMRI in patients with postoperative strabismus and in healthy volunteers. For patients with strabismus, binocular summation was less important at the foveal projection area and at the peripheral 2-degree projection area. This result suggests that abnormal cortical visual processing causes cortical suppression of the prefoveal projection area in patients with strabismus.

Visual neuropsychology

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

We tried to assessed how the degrees much a visual cortex and a visual pathway haveof plasticity and stability of the visual cortex and the visual pathways in in a patient who hads a central scotoma with a lesions on of either cones or retinal ganglion cells. BesidesIn addition to using fMRI as we have beenhave used forin the previous studies, we measured the performed patients with diffusion MRI, which allows us to quantify a the visual pathway, which that consists of white matters, based on the basis of on the free movements of water molecules. Ogawa et al. have reported the results for of quantification of the visual pathway in patients with a lesion on of either cones or retinal ganglion cells.

2. Identification of cortical area for visual awareness

The Because the human temporo-parietal junction (TPJ) is a very large cortical region and that responds to many kinds of stimuli, so the precise mapping init has not been precisely mapped TPJ is still unclear. Here, using functional fMRI and a mixture of visual and auditory stimuli, we revealed a small visually responsive area in the right temporoparietal junctionTPJ (vTPJ), which has. Our results and previously been literature suggested that vTPJto may 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 which 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-gauge and 25-gauge transconjunctival vitrectomy systems for treating macular hole, epiretinal membrane, macular edema, and rhegmatogenous retinal detachment. The 25- and 23-gauge sutureless vitrectomy techniques decrease 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 of the 20-gauge high-speed vitrectomy system, the use of 25- and 23-gauge transconjunctival vitrectomy systems may effectively reduce operative times in select cases that do not require the full capability of conventional vitrectomy.

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

To evaluate 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).

To evaluate clinical efficacy of 7 mm intraocular lens (ETERNITY® Santen Pharmaceutical Co. Ltd.) for combined pars plana vitrectomy, phacoemulsification and intraocular lens implantation, we observed the visibility of the retina during vitrectomy and measured the depth of anterior chamber preoperatively and postoperatively with the PENTACAM®.

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

We evaluated changes in regular and irregular corneal astigmatism after 25-gauge and 23-gauge transconjunctival sutureless vitrectomy.

We investigated changes in corneal thickness following vitreous surgery and determined

whether such changes can be used as a criterion for evaluating the invasiveness of vitrectomy.

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 to evaluate 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 of 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 vulnerable of retina ganglion cells in diabetes mellitus has been reported in patients 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 evaluated the changes in health- and vision-related quality of life (HR-QOL and VR-QOL) in patients with Behçet uveitis receiving infliximab therapy. In We found that infliximab therapy conclusion, relievedf of episodes of uveitis attacks and extraocular manifestations byand infliximab therapy significantly improved the HR-QOL and VR-QOLboth health- and vision-related quality of life in patients with Behçet uveitis.

2. We studied choroidal thickness (CT) and circulation (CC) before and after immunosuppressive therapy in Vogt-Koyanagi-Harada disease (VKH). In conclusion, we found that evaluation of the choroidal status by with spectral domain (SD)-OCT and indocyanine green angiography ICGA was helpful to for monitoring the therapeutic response in VKH. Furthermore, indocyanine green angiography ICGA was superior to SD-OCT in the follow-up evaluation of this study.
3. We described a patient with Behçet disease in BD whom developed MSmultiple sclerosis—like lesions developed in the CNS central nervous system and spinal cord.
This case indicates that clinicians should pay attention to the development of MS-like lesions in the CNS and spinal cord of a patient with BD.
4. We investigated the relationship between serum levels of interleukin IL-6 and disease activity in patients with Behçet diseaseBD and concluded that levels of interleukin 6 might be a marker of disease activity in Behçet disease. In conclusion, circulating IL-6 may be a marker of BD activity.

Macular degeneration
1. We reported a case of macular hole because of prolonged viewing of a plasma flash by a femtosecond laser that was followed up with spectral domain optical coherence tomography (SD-OCT).
2. We evaluated the efficacy of reduced-fluence photodynamic therapy (RFPDT) for central serous chorioretinopathy (CSC). In conclusion, we found that RFPDT appears to be an effective treatment method for CSC. Outer nuclear layer ONL thickness is an important visual predictive factor of for the effect of RFPDT for CSC.
3. We described findings in spectral-domain optical coherence tomography (SD-OCT) findings forfrom four 4 patients with acute foveal photoreceptor damage. Lesions exhibiting orange-yellow foveal granularity characteristic of these four 4 cases corresponded to hyperreflective localized photoreceptor lesions on SD-OCT. Spectral-domain optical coherence tomographySD-OCT was useful for detecting an acute disruption and a resolution of the photoreceptor layer in the fovea.
4. We described a patient with spontaneous resolution of serous foveal detachment SFD in a dome-shaped macula DSM documented bywith serial spectral domain optical coherence tomography (SD-OCT).
5. We investigated the involvement of TNFtumor necrosis factor-α and monocyte chemoattractant protein MCP-1 in the serum and peripheral blood in of patients with neovascular age-related macular degenerationAMD (nAMD). In conclusion, Wwe found a close relationship between the cell-associated of tumor necrosis factor α and monocyte chemoattractant protein 1 TNF-α and MCP-1 and neovascular age-related macular degenerationnAMD.

Biochemistry
1. The therapeutic effects of cyclosporine A encapsulated in biocompatible and biodegradable blended nanoparticles of poly (lactic acid) (PLA) homopolymers and PEGpolyethylene glycol-block—poly (lactic acid) PLA copolymers (stealth nanocyclosporine) were examined in an experimental autoimmune uveoretinitis (EAU) model in Lewis

rats. The strong therapeutic benefit of stealth nanocyclosporine on in experimental autoimmune uveoretinitis EAU obtained with the stealth nanocyclosporine may have been due to sustained release in situ, prolonged blood circulation, and targeting to of the inflamed uvea and retina, in addition to sustained release in situ.

2. We report microglial/macrophage activation in a mouse model of both a Stargardt disease and age-related macular degeneration mouse model caused by delayed clearance of all-trans-retinal from the retina, and in a mouse model of retinitis pigmentosa mouse model with impaired retinal pigment epithelium (RPE) phagocytosis. This study demonstrates an important contribution of Toll-like receptor TLR4—mediated microglial activation by endogenous photoreceptor proteins in retinal inflammation that aggravates exacerbates retinal cell death. This pathway is likely to represent an underlying common pathology in degenerative retinal disorders.

Color vision defects and genetic analysis of retinal diseases

1. Retinitis pigmentosa and its allied disorders have genetic heterogeneity. In other words, there are lots ofmany causative genes in among those these disorders. Although direct sequencing analysis for several causative genes ishas generally been performed, there are few cases to identify causative gene mutations. So therefore, we have performed chose whole-exome sequencing analysis to identify gene mutations. With this method, we 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 cone dystrophies. We identified causative mutations in those these diseases. To clarify disease haplotypes, the results of haplotype analysis with mutations was 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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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 vestibu-

lar 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.

For the selection of astronauts by the Japan Aerospace Exploration Agency, our neuro-otology team performed third-stage examinations at the Tsukuba Space Center. In this examination, the candidates' aptitude to be astronauts was tested by applying Coriolis stimulation with a rotating chair to provoke motion sickness.

Research in rhinology

We are involved in the analysis of data on factors related to the intractability of rhinosinusitis obtained from patients undergoing endoscopic sinus surgery (ESS) and from prospective studies of the postoperative course. We perform special care for skull base diseases, such as pituitary tumors and CSF leak, with a good relationship with the Department of Neurosurgery. We report case studies and investigate the postoperative course of skull base diseases. In an attempt to expand the indications for ESS from paranasal sinus tumors to skull-base surgery, including that for spinal fluid leakage, skull-base tumors, and pituitary gland tumors, and to improve the safety of ESS, we have performed high-tech navigation surgery in which 3-dimensional endoscopic images and stereonavigation images are superimposed. 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 cadavers at the Skills Laboratory, for both skull-base surgery and endoscopic sinus surgery training. We must improve both medical techniques and anatomical knowledge. In addition, 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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Takahiro Matsumoto, *Assistant Professor*
Kazuhiro Shoji, *Assistant Professor*
Gumi Hidano, *Assistant Professor*
Hiroshi Sunaga, *Assistant Professor*
Sachiko Omi, *Professor*
Shuya Kiyama, *Professor*
Masaki Kitahara, *Associate Professor*
Ichiro Kondo, *Associate Professor*
Shigehiko Uchino, *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 2013 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 2013.

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: cardiovascular anesthesia, thoracic anesthesia, pediatric anesthesia, regional anesthesia, neuroanesthesia, obstetric anesthesia, intensive care medicine, and comprehensive pain management. Faculty recruitment is targeted at individuals with demonstrated academic and research activities as well as excellent clinical management and teaching skills. In 2013 Dr. Shimoyama, who was recruited to renew the palliative care program, successfully established a comprehensive palliative care team for cancer patients, a first for The Jikei University, in close collaboration with our existing outpatient pain clinic and acute pain service. Our chronic pain clinic, led by Dr. Kitahara, was recognized by the Ministry of Health, Labour and Welfare as a tertiary pain center in Japan. The members of our pain clinic are expected to play a leading role in establishing various guidelines for pain management in Japan.

Our faculty and residents were both well represented at the Japanese Society of Anesthesiologists' annual meeting in Sapporo and the American Society of Anesthesiologists' annual meeting in San Francisco. In addition, members of the department continue to be invited as visiting professors or guest speakers at national and international meetings. Listed below are the ongoing research projects in which the principal investigators are faculty members of the Department of Anesthesiology.

Doctor Hobo found that gabapentin augments the therapeutic effect of valproic acid on postoperative pain, partly through a mechanism involving the glutamic transporter at the spinal cord level. These experimental findings were further supported by a preliminary clinical trial performed by Dr. Hobo and his colleagues. Doctor Mio's research has focused on the effects of mitochondria on the preservation of major organs. He found that volatile anesthetic agents have protective effects on mitochondria in renal cells. While nicorandil was believed to be protective against contrast-induced kidney injury, Dr. Mio found that nicorandil showed no protective effect against mitochondrial dysfunction in renal cells exposed to a contrast agent. Doctor Kondo continued to work on tumor proliferation induced by morphine. He observed that morphine's proliferative effects on tumor cells are not dose-dependent.

In clinical medicine, several principal investigators from the Department of Anesthesiology deserve mention. Doctor Taniguchi has been interested in temperature regulation during surgery and its effects on postoperative outcomes, such as shivering. Doctor Uchino continues to be active in clinical research in the intensive care unit (ICU) and has been extremely productive in the field of acute kidney injury. Using a large database in the intensive care unit, Dr. Uchino and his colleagues demonstrated that low molecule hydroxyethyl starch currently used in Japan does not have any effect on postoperative bleeding. His group reviewed several predictive factors associated with readmission to the ICU of patients who were admitted to the ICU for an overnight stay with monitoring after surgery. They found that the 2 major risk factors for readmission were administration of a large volume of fluids in the operating room and postoperative ventilation.

The physicians of our pain clinic continue to play a pivotal role in establishing practice guidelines for patients with neuropathic pain. Their particular interest has been post-mastectomy 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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Department of Rehabilitation Medicine

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Kun Suk Chung, *Assistant Professor*
Itaru Takehara, *Assistant Professor*
Tadashi Suzuki, *Assistant Professor*
Keiji Hashimoto, *Assistant Professor*
Shu Watanabe, *Professor*
Wataru Kakuda, *Associate Professor*
Hideki Sugawara, *Assistant Professor*
Masanori Funakoshi, *Assistant Professor*
Nobuyuki Sasaki, *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) botulinum toxin type A (BoNT-A) for stroke, 3) development scales for children, 4) evaluation of dysphagia, 5) prevention of hospitalization-associated disability, and 6) traumatic brain injury (TBI).

Research Activities

rTMS for stroke

1. Functional cortical reorganization after low-frequency rTMS plus intensive occupational therapy for upper limb hemiparesis: evaluation by functional magnetic resonance imaging in patients after stroke

The results indicated that our proposed treatment can induce functional cortical reorganization, leading to motor functional recovery of the affected upper limb. In particular, neural activation in the lesional hemisphere appears to play an important role in such recovery in patients with hemiparesis after stroke.

2. Bihemispheric rTMS combined with intensive occupational therapy for upper limb hemiparesis after stroke: a preliminary study

The combination of bihemispheric rTMS at both 1 and 10 Hz and intensive occupational therapy was both safe and feasible and improved the motor function of the hemiparetic upper limb in patients after stroke.

3. High-frequency rTMS using a double-cone coil for gait disturbance

High-frequency rTMS of bilateral leg motor areas using a double-cone coil can potentially improve walking function in patients with hemiparesis after stroke.

4. High-frequency rTMS applied over bilateral leg motor areas combined with mobility training for gait disturbance after stroke: a preliminary study

The protocol featuring high-frequency rTMS with a double-cone coil and mobility training is safe and feasible and can improve walking function after stroke.

5. Bilateral rTMS combined with intensive swallowing rehabilitation for chronic stroke dysphagia: a case series study

A 6-day protocol of bilateral rTMS combined with intensive swallowing rehabilitation improved laryngeal elevation delay time in all patients. Our proposed protocol of rTMS plus swallowing rehabilitation exercise seems to be safe and feasible for chronic dysphagia after stroke.

BoNT-A for stroke

1. Long-term effects of injection of BoNT-A combined with home-based functional training for patients with spastic upper limb hemiparesis after stroke

The combination of BoNT-A injection and home-based functional training reduced spasticity and improved the motor function of the proximal upper limb and the fingers.

2. Clinical efficacy of a double-injection protocol of BoNT-A for upper limb hemiparesis after stroke

A more significant improvement was found in muscle spasticity and in upper limb motor function after 2 injections of BoNT-A. Our findings suggest that repeated injections of BoNT-A followed by a comprehensive rehabilitative program would be an effective treatment for limb spasticity after stroke.

Development scales for children

1. Validity of the Family-Rated Kinder Infant Development Scale for Children

This study provides evidence for the validity of the family-rated Kinder Infant Development Scale for assessing the developmental age of children in early childhood.

Evaluation of dysphagia

1. Applicability of the 2-step thickened water test in patients with poststroke dysphagia: a novel assessment tool for paste food aspiration

The 2-step thickened water test might be a useful tool for assessing the risk of paste food aspiration in patients with dysphagia after stroke.

Prevention of hospitalization-associated disability

1. Systematic introduction of a system to prevent hospitalization-associated disability: preliminary trial to improve the quality of medical care for hospital patients

Our proposed system to prevent hospitalization-associated disability was safely introduced in our hospital and appears to facilitate the early rehabilitation of hospitalized patients.

TBI

1. Vocational rehabilitation for clients with cognitive and behavioral disorders associated with TBI

Despite a high prevalence of cognitive and behavioral disorders after moderate-to-severe TBI, long-term functional improvement is likely to occur in clients with TBI. Greater gains in both physical and cognitive functions are made through a multidisciplinary, wide-ranging, comprehensive approach to rehabilitation.

Publications

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Department of Emergency Medicine

Takeki Ogawa, *Professor*
Satoshi Takeda, *Associate Professor*
Kei Ohtani, *Associate Professor*
Taro Nameki, *Assistant Professor*
Tsutomu Koyama, *Professor*
Masahiko Uzura, *Associate Professor*
Kenji Dohi, *Associate Professor*
Kenji Okuno, *Assistant 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 of traumatic brain injury
9. Basic and clinical researches of oxidative stress and emergency medicine
10. Advice to lacal suthorities on plans for disaster medicine
11. Creation of DMAT (Disaster Medical Assistance Team) deployment system
12. Management of hospital emergency response drill

Research Activities

1. Supervision and development of ultrasound devices in the diagnosis and treatment of cerebrovascular disorders
2. Director of Japan Neurotrauma Data Bank Committee
3. Prognostic value of heart fatty acid-binding protein for patients with chest symptoms in the emergency room
4. Research committee on higher cerebral function after traumatic brain injury
5. Research committee on impact biomechanics in automobile accidents (Society of Automotive Engineers of Japan, Inc.)
6. Published a revised edition of *Guidelines for the Treatment and Management of Severe Head Injury* (The Japan Society of Neurotraumatology)
7. Research group on cerebospinal fluid in cases of traumatic intracranial hypotension
8. Management of the Japan Advanced Trauma Evaluation and Care Course
9. Basic research of traumatic brain injury and oxidative stress
10. Basic research of heat stroke and neuronal injury
11. Development of anti free radical therapy in patients with acute neuronal conditions

Publications

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Department of Endoscopy

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General Summary

Our main area of research is performing clinical studies of endoscopy in 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), narrow narrow-band 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. Our research utilizes the following 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 is particularly useful for screening patients from the medical checkup population, as it will reduce discomfort during endoscopic examination.

1) Magnifying endoscopic observation using a narrow-band imaging system

This new diagnostic system consists of a magnifying (×90) endoscope and an NBI light source, which provides detailed morphological information about capillaries on the mucosal surface. We studied the clinical utility of NBI magnifying endoscopy for superficial neoplasms in the pharynx, esophagus, stomach, and duodenum. One study focused on the development of algorithms for NBI technology which would allow the histological type and tumor extent of gastric carcinoma to be determined without biopsy. On the basis of our findings with magnified NBI, we have also developed a novel classification system for gastric cancer and demonstrated, in a prospective study, its advantages over the conventional diagnostic system. We 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 comparing NBI magnifying endoscopy with Lugol chromoendoscopy for detecting superficial carcinoma in the esophagus. We also

aim to evaluate this technology for the early detection of precancerous changes in the specialized columnar epithelium of Barrrett's esophagus. Results of these studies have been reported at several conferences and published in several English-language journals.

2) Endocytoscopy

Endocytoscopy is a novel optical imaging technique that allows the gastrointestinal mucosa to be visualized in vivo and in real time at the cellular level using a staining solution. We joined a multicenter study using endocytoscopy for diagnosing superficial esophageal squamous cell carcinoma. Moreover, 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.

3) AFI

Recently, the AFI endoscopic system has been developed to endoscopically visualize autofluorescence emitted from the gastrointestinal wall. Theoretically, AFI can be used to detect premalignant lesions or early-stage malignancies that do not have a distinct appearance on conventional white-light endoscopy. Although AFI is still associated with a high false-positive rate, we established that AFI, in combination with conventional white-light imaging and NBI, can improve specificity. This result was published in an English-language journal.

4) Ultrathin endoscopy (transnasal endoscopy)

Ultrathin endoscopy can reduce discomfort during endoscopic examination. However, the ultrathin endoscope has a poorer image resolution than do conventional endoscopes, and, therefore, has a higher risk of false-negative results. Accordingly, we found that ultrathin endoscopy was less able to detect gastric lesions than was high-resolution endoscopy. We are now attempting to develop 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 histopathological analysis of lesions that are usually 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 to precisely guide the biopsy needle into lesions. The tissues obtained with EUS-FNA are immediately examined by a cytologist or pathologist to detect the presence of malignant cells. We are now evaluating the technical safety and usefulness of this technique in ongoing studies.

2. Endoscopic treatment of esophageal and gastric malignancies

With recent advances in endoscopic diagnostic techniques and instrumentation, indications have expanded for endoscopic therapy in early gastric and esophageal carcinomas. Research on the following endoscopic therapeutic modalities is now under way to standardize the use of these techniques 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 the use of 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 also evaluated the effectiveness of gastric acid-suppressing drugs, which have been used empirically after endoscopic treatment, by monitoring intragastric pH after endoscopy. We have also used blood cultures to study the risk of sepsis and endotoxemia following 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 usually difficult to access. We have previously reported on the use of the M-scope in the treatment of tumors of the lesser curvature, greater curvature, and posterior wall of the gastric body, and the cardiac region, which are not accessible with conventional endoscopes. Furthermore, clinical studies using a newly developed therapeutic endoscope (R-scope), which has a special mechanism allowing the forceps to move laterally and vertically, in addition to the multibending function, are proceeding to advance the potential of endoscopic therapy. We have also performed several studies using natural orifice translumenal endoscopic surgery, including full-thickness resection, because current endoscopic treatments are directed only at mucosal diseases.

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

Many studies have demonstrated an association between *H. pylori* infection and the development of gastric cancer. However, there are still many unknown factors affecting this association. Because our department routinely performs endoscopic treatment for gastric cancer, clarification of these factors is important. Experiments concerning this association, particularly on 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 roles of inducible nitric oxide synthase (iNOS) in the pathogenesis of *H. pylori*-associated diseases and have demonstrated that eradication of *H. pylori* plays an important role in the process of repairing disease-associated DNA methylation and in the alteration of methylation patterns of genes in the mucosa in the 5 years following *H. pylori* eradication. Interim results have been reported at several conferences and been published in Japan as well as internationally. In addition, we have reported that diverse topographical patterns of *H. pylori*-induced iNOS expression and iNOS gene polymorphism may contribute to the development of gastric cancer caused by *H. pylori* infection.

4. Diagnosis of oropharyngeal and hypopharyngeal malignancies

Endoscopic screening with iodine staining, or Lugol chromoendoscopy, has enabled esophageal cancer to be detected at an early stage and, thus, has improved prognoses. However, this technique is difficult to perform in such locations as the oropharynx or hypopharynx. Metachronous or synchronous cancer of the oropharynx or hypopharynx has become the main factor adversely affecting the prognosis and quality of life of patients with esophageal cancer. Because detecting cancer at an early stage is important, we have found that magnifying endoscopy in combination with the NBI system has allowed hard-to-find cancers to be detected during the early stages without the need for

Lugol chromoendoscopy. A multicenter randomized controlled study of the clinical value of this new combination endoscopy was performed. In addition, we performed a single-center study to evaluate 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 choosing effective treatments. Hence, we have developed a new method of evaluating esophageal functions using a small-caliber endoscope. We have started basic experiments on esophageal motility and sensitivity, with the aim 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 clarified several of the factors that increase the likelihood that esophagogastric varices will recur 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 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 unreachable with an ordinary endoscope system. Internationally, capsule endoscopy has been performed in more than 1 million cases 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 single-balloon enteroscopy, which allows biopsy and hemostasis to be performed for hemorrhagic lesions of the small intestine.

The numbers of cases of colonic cancer have increased markedly in Japan. In Europe and the United States, several studies have described the use of capsule endoscopy for examining the large intestine. In Japan, we are collaborating with another hospital to perform studies with capsule endoscopy to screen patients for colonic neoplasms.

Accurate preoperative evaluation of the degree of tumor invasion into deep layers 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 utilize a magnifying endoscope with NBI and crystal-violet staining or 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, endoscopic resection of large intestinal lesions is technically difficult because of the wide lumen and the higher rate of complications, such as perforation and bleeding. Our present efforts are focused on establishing safe and reliable methods to remove large colonic lesions endoscopically and to start preliminary use of ESD. Additionally, an infrared endoscopy system has been used to evaluate the risk of bleeding from vessels located at the base of the ulcer created with ESD.

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 have been unreachable 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 289 patients since the Japanese health insurance system began covering this procedure in April 2007. Our study found that capsule endoscopy should be performed as soon as possible following a patient visiting hospital with a complaint of melena. We are aiming to further 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 a nonneoplastic tumor of the colon. Therefore, endoscopic treatment is not indicated on a histologic basis. However, according to recent reports in Western countries, sessile serrated lesions can develop into advanced, invasive submucosal cancers that invade deeper layers. We are now studying the use of the AFI and NBI systems to detect neoplastic lesions, including sessile serrated polyps, and are examining biological markers of malignancy in sessile serrated lesions by means of immunohistochemical staining to evaluate whether such these lesions have malignant potential.

Submucosal invasive cancer, which invades to a depth of less than 1,000 μm, is an indication for endoscopic treatment according to the 2010 Guideline for the Treatment of Colorectal Cancer from the Japanese Society for Cancer of the Colon and Rectum. Therefore further investigation with magnifying endoscopy is necessary. We are studying the characteristic findings of conventional and magnifying endoscopy. In cases in which the submucosal invasion depth is the only component 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. A research project including our hospital concerning the stratification of risk factors for the metastasis of deep submucosal invasive cancers (invasion depths >1,000 μm) to other organs is under way.

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. In additional we introduced second-generation contrast media for ultrasonic imaging in the EUS diagnosis of pancreaticobiliary diseases.

We are also developing new diagnostic markers for pancreatic carcinoma and a system for measuring them, and we will be applying these markers to the differential and prognostic diagnosis of pancreatic carcinoma using specimens obtained with EUS-FNA.

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 post-ERCP pancreatitis, a common complication.

2. Treatment using endoscopic techniques in pancreatobiliary diseases

The technique of EUS-guided celiac plexus block has been performed to control persistent pain due to chronic pancreatitis, even in benign disease. We have performed EUS-guided celiac plexus neurolysis using a small amount of injected ethanol and are now evaluating the feasibility of this approach.

We have also started animal experiments to develop new interventional technologies to locally control pancreatic cancer and to diagnose gallbladder neoplasms.

Palliative care

More and more interest is being shown in palliative care. 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. Although percutaneous endoscopic enterostomy is conventionally not 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 technique's clinical usefulness in this situation. Kits for percutaneous endoscopic gastrostomy developed by us have reduced the frequency of complications associated with percutaneous endoscopic enterostomy placement. To alleviate stenosis attributable to tumors of the digestive tract and bile duct, we have performed endoscopic ballooning/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 contribute to improving the quality of life of patients who are not candidates for radical surgery. The cost-effectiveness of these interventions is another benefit.

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Department of Infection Control

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General Summary

We performed clinical research on urinary tract infection and bacteremia caused by extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*, *Streptococcus* sp., and methicillin-sensitive *Staphylococcus aureus* (MSSA) as bacterial infections. Our results suggest methods for the appropriate diagnosis and treatment of these infectious diseases to all physicians. In addition, we showed the efficacy of nontechnical skills for infection control that suggested a new strategy independent of any equipment. We will extend these investigations to basic research and large prospective studies.

Research Activities

Prevalence and drug susceptibilities of ESBL-producing E. coli strains isolated from urine

We investigated the prevalence and drug susceptibilities of ESBL-producing *E. coli* isolated from urine and the genotyping of ESBL to clarify strategies against urinary tract infections caused by these bacteria. From March 2010 through June 2012 ESBL-producing *E. coli* were isolated from 41 patients in The Jikei University Katsushika Medical Center. Their median age was 76 years. Nineteen patients (46.3%) had community-acquired infections. Forty-one strains of ESBL-producing *E. coli* were detected. The minimal inhibitory concentrations of 17 antimicrobials — latamoxef, flomoxef, cefmetazole, imipenem, meropenem, doripenem, faropenem, sulbactam/ampicillin, clavulanic acid/amoxicillin, tazobactam/piperacillin, sulbactam/cefoperazone, levofloxacin, ciprofloxacin, sitafloxacin, gentamicin, amikacin, and tobramycin — against these strains were measured with the broth microdilution method according to the Clinical and Laboratory Standards Institute. In addition, genotyping of ESBLs was performed with the polymerase chain reaction. Results of genotyping of ESBLs were as follows: the CTX-M-9 group, 31 strains (75.6%); the CTX-M-1 group, 6 (14.6%); and the CTX-M-2 group, 4 (9.8%). All strains were sensitive to meropenem, doripenem, imipenem, tazobactam/piperacillin, latamoxef, flomoxef, cefmetazole, faropenem, and amikacin, and 73% of these strains were sensitive to sitafloxacin. However, 73.2% of these strains were levofloxacin-resistant. These results indicate that ESBL-producing *E. coli* are frequently resistant to levofloxacin. Therefore, in the future we should pay attention to the choice of antimicrobials for urinary tract infections caused by ESBL-producing *E. coli* and continuously examine their drug susceptibilities.

Teaching nontechnical skills is effective for infection control

We used TeamSTEPPS (Team Strategies and Tools to Enhance Performance and Patient

Safety), a popular educational tool for nontechnical skills, to teach infection-control compliance to healthcare workers in The Jikei University Hospital in 2013. In particular, "cross-monitoring and feedback" were applied to develop hand-hygiene compliance in each hospital unit. After these efforts, the total consumption of alcohol hand rub showed a 29.6% increase from the previous year. The teamwork tools of TeamSTEPPS are useful for developing infection-control compliance in the hospital.

Predictive factors for metastatic infection in patients with bacteremia caused by MSSA
Metastatic infections, such as infective endocarditis and psoas abscess, are serious complications of *S. aureus* bacteremia, because failure to identify these infections may result in bacteremia relapse or a poor prognosis. In the present study, we determined predictive factors for metastatic infection due to MSSA bacteremia. A retrospective cohort study was performed among patients with MSSA bacteremia at The Jikei University Hospital from January 2008 through December 2012. Factors analyzed included the underlying disease, initial antimicrobial treatment, and primary site of infection. During the 5-year study period, 73 patients met the inclusion criteria and were assessed. The most common primary site of bacteremia was catheter-related bloodstream infection (25 of 73 patients [34.2%]). Metastatic infection occurred in 14 of 73 patients (19.2%) (infective endocarditis, 3 patients; septic pulmonary abscess, 3 patients; spondylitis, 4 patients; psoas abscess, 4 patients; epidural abscess, 3 patients; and septic arthritis, 1 patient). Six patients had multiple metastatic infections. Multivariate analysis revealed that the predictive factors associated with the development of 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 in the 2 weeks after the onset of bacteremia. This study demonstrated that additional diagnostic tests should be performed to identify metastatic infection, particularly in patients with delayed antimicrobial treatment, persistent fever, and persistently high C-reactive protein levels.

Analysis of the components of the biofilm formed by clinical strains of staphylococci
The components of the biofilm formed by staphylococci are polysaccharides, proteins, and extracellular DNA. These biofilms are thought to be composed of components in complexes or alone. In the biofilm formation assay, staphylococci formed a biofilm rich in polysaccharides with the addition of 4% NaCl to brain heart infusion broth. This is presumably due to the high osmotic pressure in the medium. The pH of the medium was lowered with the addition of 1% glucose, and proteins were the main components of the biofilm. By means of this phenomenon, clinically isolated staphylococci were divided into non-biofilm-forming strains, polysaccharide biofilm-forming strains, and proteinaceous biofilm-forming strains. In the biofilm destruction assay, we tested a variety of enzymes to destroy the biofilms formed by staphylococci. Proteinase K destroyed the proteinaceous biofilms, and polysaccharide biofilm were susceptible to dispersin B. On the other hand, the susceptibilities to DNase were nonidentical in clinical isolates.

Acquired immunodeficiency syndrome and malignancy

Although infection with the human immunodeficiency virus (HIV) has become a controllable chronic illness because of improvements in antiretroviral agents, complications not related to acquired immunodeficiency syndrome (AIDS), such as cardiovascular diseases, dementia, and malignancy, have become major problems. Of these complications, malignancy is an important and critical issue.

Malignancies in patients with HIV were classified into 2 types: AIDS-defining malignancies (ADMs) and non-AIDS-defining malignancies (NADMs). Recently, NADMs are more frequent than ADMs, the management of NADMs matters currently. The most common NADMs are Hodgkin lymphoma, lung cancer, cervical/anal cancer, and hepatocellular carcinoma.

The clinical course of malignancy in patients with HIV is generally aggressive, and most cases are difficult to treat. However, early diagnosis and radical surgical resection improve the prognosis. Therefore, we must be concerned about primary and secondary prevention of malignancy as well as the treatment of AIDS. Primary prevention measures that should be implemented include the control of co-infection by oncogenic viruses, the avoidance of exposure to environmental oncogenic factors, and the earlier start of antiviral therapy. As a secondary prevention measure, medical checkups similar to those for patients without HIV are recommended for all patients with HIV.

In our hospital, we treated a patient with AIDS who had invasive thymoma and another patient who had penile cancer, the surgical treatment of which resulted in cure without relapse. Early diagnosis and treatment of malignancy might contribute to good outcomes in patients with HIV.

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Department of Dentistry

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General Summary

1. Clinical studies of temporomandibular disorders

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

2. Morphological and histological studies of the temporomandibular joint

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 examine the relationship between body-mass index (BMI) and the fatty change of the suprahyoid muscles in patients with obstructive sleep apnea (OSA).

Research Activities

Clinical studies of TMDs

1. Time trend for clinical background factors of patients with TMDs at general clinical offices in metropolitan Tokyo

Purpose: We have developed a 4-item questionnaire to screen patients for TMDs, and a portion was used for a Ministry of Health, Labour and Welfare survey of dental diseases, and with the cooperation of the Tokyo Dental Association, we performed a questionnaire survey of applicants for dental checkups at general dental offices in Tokyo. We reported personal computer (PC) duties and their connection with TMDs in 2011 and 2012. In 2007, 2009, and 2012, we performed a questionnaire survey with the Tokyo Dental Association. On the basis of these 3 investigations, we reviewed annual transition of the PC operation times when they were thought to affect results of the TMD screening test.

Methods: We analyzed secondary data of the Tokyo Dental Association for 2007 (180 subjects: 101 men and 79 women) and 2009 (76 subjects: 54 men and 22 women), and 2012 (69 subjects: 13 men and 56 women).

Results: "PC operation time" increased significantly each year (Kruksal-Wallis analysis). We also recognized a difference in mean values among the 3 groups (Mann-Whitney test). The effect size (r) showed an appropriate value. The Spearman correlation coefficient showed weak negative correlations of "PC operation time" with "long time before going to bed" and "sleep time."

Conclusion: The increasing "PC operation time" each year and the time of going to bed shortening after return suggest that further investigation is necessary.

2. Epidemiological characteristics of patients with TMDs in Japan

Purpose: In 2007, Sugisaki et al. developed a 4-item questionnaire for screening patients for TMDs: it showed a sensitivity of 0.746 and a specificity of 0.811. In the same year,

they reported the validity of a single binary scale (yes/no) question: “When you open your mouth widely and/or close it, do you feel pain in your jaw?” from the 4-item questionnaire (sensitivity, 0.701; specificity, 0.871). In this study, we examined the epidemiological characteristics of patients with TMD in Japan using the TMD screening questionnaire mentioned above.
Methods: We analyzed several epidemiological reports: 1,071 people working in Tokyo in 2005 (study 1), 1,969 people working at a single company assessed by Nishiyama et al. in 2007 (study 2), the National Survey of Dental Diseases in 2005 and 2012 (study 3), and the National Adult Dentistry Health Investigation in 2007 (study 4), which used the same TMD screening items.
Results: The percentages of persons complaining of jaw pain were 3.4% (in 2005) and 3.3% (in 2012) in study 3 and 3.5% in study 4. The rates of jaw pain found in study 1 for people working in the Tokyo metropolitan area were 17.9% with the 4-item questionnaire (in 2005 and 2006) and 20.0% with the single binary-scale question (in 2006). Moreover, the rate in study 2 was 22.6%.
Conclusion: The prevalence of TMD in employed subjects is higher than that of the general public.

Anatomical studies of the TMJ of marsupials

1. Absence of the articular disk in the Tasmanian devil TMJ

Purpose: The articular disk of the TMJ is a conserved structure in mammals. According to Parsons’ report in 1900, however, the articular disk is absent in 4 animals: the armadillo, 2 kinds of monotreme, and the Tasmanian devil. Thereafter, no research was performed to confirm this observation. The aim of this study was to determine with anatomical and histological examination whether the Tasmanian devil TMJ has an articular disk.
Methods: Six fresh-frozen corpses and 1 dry skull of Tasmanian devils were obtained from the School of Zoology, University of Tasmania. The corpses were dissected, and the morphology of the TMJ was carefully observed through gross anatomical and histological examination. The structure of the TMJ of the dry skull was examined macroscopically and with micro-computed tomography (CT).
Results and Conclusion: In all cases, absence of the articular disk in the Tasmanian devil TMJ was morphologically confirmed. The surface layer of both the condyle and the glenoid fossa comprised thick fibrous tissue. Micro-CT revealed dense and fine trabecular bone in the condyle. The thick fibrous tissue covering the condyle and high-density trabecular bone in the condyle might play a role in absorption against powerful mastication and the heavy loading of the Tasmanian devil TMJ.

Clinical studies of OSA-hypopnea syndrome

1. The effect of fatty changes in the lingual muscles and BMI on the apnea-hypopnea index

Purpose: A change in muscle function has been postulated to be associated with the etiology of OSA. Saito and colleagues have reported on 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, on the basis of previous images, fat-to-muscle metamorphosis has not been shown in humans. Here, we show evidence of fatty metamorphosis in the lingual muscles in CT images of patients with suspected OSA.

Methods: The subjects were 62 patients (47 men and 15 women) with suspected OSA who had visited the Tsurumi University School of Dental Medicine from November 2007 through October 2011. All subjects gave informed consent to take part in the study. Subjects underwent CT evaluations at the imaging diagnosis department of the hospital. Sex, age, BMI, and the apnea-hypopnea index (AHI) were recorded for each patient. Inferior airway space and the total value of the length and width of the inferior airway space (TIAS) were also measured. The degree of fat-to-muscle metamorphosis was measured with CT. Image-analysis software (Aze Win, Aze Ltd., Tokyo, Japan) was used to set the regions of interest (ROIs) of 30 mm^2 on the bellies of the lingual muscles. We measured CT levels of 4 ROIs (both sides of the central area and both sides of the posterior area) in the genioglossus muscles and 2 sizes of ROI (both sides of the central area) in the geniohyoid muscles. Values were quantified and compared statistically.

Results: The median values (and 25% and 75% quartile deviations) were as follows: patient age, 51.50 years (42.75, 62.25); BMI, 24.00 kg/m^2 (22.00, 26.00); AHI, 24.35 (11.40, 36.10), genioglossus CT levels, 123.05 (90.95, 135.70); geniohyoid muscle CT levels, 111.20 (104.80, 116.30); and TIAS, 34.65 (25.97, 40.62). Analysis of the results of a multiple regression model with Amos (Version 6) software (Amos Development Corporation, Spring House, PA, USA) showed that the standardized estimates of the BMI were -0.50 ($p = 0.000$) for the genioglossus muscle and -0.42 ($p = 0.000$) for the geniohyoid muscle. The standardized estimate of BMI of the distance of the TIAS was -0.55 ($p = 0.001$), and that of the AHI of the TIAS was -0.48 ($p = 0.000$).

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

2. The relation of fatty changes in lingual muscles and BMI with high AHI

Introduction: A change in muscle function has been postulated to be associated with the etiology of OSA. At the 17th annual meeting of the Japanese Society of Dental Radiology we reported fatty changes in the lingual muscles of a patient with suspected OSA. We have shown evidence of fatty metamorphosis in the lingual muscles and its relation to BMI using CT images of patients with high AHI (more than 30) and diagnosed OSA.

Methods: The subjects were 26 patients with high AHI from among 62 patients (47 men and 15 women) with suspected OSA who visited the Tsurumi University School of Dental Medicine from November 2007 through October 2011. All subjects gave informed consent to take part in the study. Subjects underwent CT evaluations at the image diagnosis department of the hospital. Sex, age, BMI, and AHI were recorded for each patient. The degree of fat-to-muscle metamorphosis was measured with CT. Image analysis software (Aze Win) was used to set ROIs of 30 mm^2 on the bellies of the lingual muscles. We measured CT levels of 4 ROIs (both sides of the central area and both sides of the posterior area) in the genioglossus muscles and two sizes of ROI (both sides of the central area) in the geniohyoid muscles. Values were quantified and compared statistically. Using the total value, BMI and fat examined relations with becoming it.

Results: The median values (25% and 75% quartile deviations) were as follows: patient age, 58.00 years (48.75 and 70.00 years); BMI, 25.00 kg/m^2 (23.00 and 28.00 kg/m^2); genioglossus CT levels, 111.05 Hounsfield units (HU) (82.97 and 134.60 HU); and geniohyoid muscle CT levels, 106.05 HU (96.47 and 116.10 HU). Analysis of the results of a multiple regression model with Amos (Ver. 6) software showed that the standardized estimates of the BMI were −0.43 (p = 0.006) for the genioglossus muscle and −0.55 (p = 0.000) for the geniohyoid muscle.

Conclusions: Our results show relationships of the fatty metamorphosis of lingual muscles and BMI with high AHI.

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Department of Transfusion Medicine

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General Summary

1. To establish guidelines that could help distinguish transfusion-related acute lung injury (TRALI) from transfusion-associated circulatory overload (TACO), a study group (T. Tasaki, principal investigator) was formed with a Health and Labour Science Research Grant last year. In the second year of this research, the proportion of donors with antileukocyte antibodies among female platelet donors was shown to be 11.7%. However, the exact proportion of patients exhibiting respiratory distress among those receiving such antibody-containing blood products remains unclear, although antileukocyte antibodies are considered a causative factor of TRALI. Furthermore, no significant difference in SpO_2 was found on the basis of whether the platelets patients received contained or did not contain antileukocyte antibodies. However, because we did show a lack of distress in patients receiving platelets without HLA-matching antibodies, more detailed analysis of the characteristics of antibodies, such as specificity and titer against the recipients' HLA phenotype, should be carried out.
2. To improve the safety of blood transfusion at the bedside, the College of Transfusion Nurses was established in Japan in 2011. Because 3 years have passed since its establishment, we sent a questionnaire of 46 questions to all 284 certified transfusion nurses (CTNs) to survey the changes in their working circumstances. 135 of 284 CTNs (47.5%) replied. The most pronounced change was that 66 of 135 CTNs (48.8%) became involved in transfusion education. Ninety-three of 135 CTNs (68.9%) felt they have improved their teaching skills for patients having transfusions.
3. In Japan, transfusions are performed even in small hospitals. For the safety of blood transfusion, accurate knowledge of transfusion medicine is necessary for physicians, nurses, and medical laboratory technologists. However, many trainees receive an insufficient education because of a lack of resources. A practical guide was created by the Study Group on Transfusion Reaction Monitoring Systems in Medical Institutions. Educating hospital staff with this practical guide will promote a higher level of transfusion operations, which will subsequently lead to improved patient outcomes. (Fujii Y, et al, Yamaguchi University)

Research Activities

1. In almost 30% of patients with TRALI, antileukocyte antibodies are found in the blood products used for their treatment. Therefore, while establishing new guidelines, we have evaluated the risk of distress due to transfusion of such blood. The subjects were patients who had received platelets derived from female donors. One sealed segment from each platelet product was frozen and screened for antileukocyte antibodies with LABScreen Multi test (One Lambda, Inc., Canoga Park, CA, USA). If the result

was positive, the specificities of the antibodies were identified with the LABScreen Single Antigen assay (One Lambda, Inc.). After informed consent was obtained, HLA typing was performed with the blood of patients who had received platelets containing antileukocyte antibodies. The relationship between antileukocyte antibodies in the donor blood and dyspnea in the recipients was investigated. The SpO_2 levels were checked before and 6 hours after the transfusion of platelets. Of the 282 specimens, 33 (11.7%) were positive for antileukocyte antibodies. The HLA typing of 3 recipients has shown that none carried an HLA phenotype corresponding to the specificities of the antileukocyte antibodies. The mean SpO_2 was reduced more in patients who had received platelets containing antileukocyte antibodies than in patients who had received platelets without such antibodies; however, the difference in antibody levels was not statistically significant. Studies of the relationship between adverse events caused by donors' antileukocyte antibodies and recipients' HLA phenotype are now underway.

2. Although the system of the College of Transfusion Nurses was only recently established, CTNs possess a large body knowledge of blood transfusion, including the management of adverse reactions, transfusion testing, and patient care, and have already improved the safety of transfusion at the bedside. We believe that the safety of blood transfusion in Japan will be improved in the future by the introduction of this new system in cooperation with transfusionists and technologists who have already been certified. (Tasaki T, et al. 24th Regional Congress of the International Society Blood Transfusion, Kuala Lumpur, Malaysia, 2013)

3. A practical guide to prevent transfusion errors in hospitals was created by the support of a Health and Labour Science Research Grant for Research on Regulatory Science of Pharmaceuticals and Medical Devices. This guide was written in both English and Japanese. Many figures and tables are used to aid understanding of the text. The guide can bee seen at the Website of the Japan Society of Transfusion Medicine and Cell Therapy. (Fujii Y, Tasaki T, et al. AABB annual meeting, Denver, 2013)

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Odaka C[1] , Kato H[2], Otsubo H[1], Takamoto S[2], Okada Y[4], Taneichi M[1], Okuma K[1], Sagawa K[3], Hoshi Y, Tasaki T, Fujii Y[4], Yonemura Y[5], Iwao N[6], Tanaka A[7], Okazaki H[8], Momose SY[8], Kitazawa J[9], Mori H[10], Matsushita A[11], Nomura H[12], Yasoshima H[13], Ohkusa Y[1], Yamaguchi K[1], Hamaguchi I[1] ([1]Nat Inst Infect Dis, [2]Aichi Med Univ, [3]Kurume Univ, [4]Yamaguchi Univ, [5]Kumamoto Univ, [6]Univ Yamanashi, [7]Tokyo Med Univ, [8]Jpn Red Cross, [9]Kuroishi Hosp, [10]Minamitama Hosp, [11]Shibetsu City Hosp, [12]Sanraku Hosp, [13]Yao Hosp). Online reporting system for transfusion-related adverse events to enhance recipient haemovigilance in Japan: a pilot study. *Transfus Apher Sci.* 2013; **48:** 95-102.

Institute of DNA Medicine
Department 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 research for 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 research for 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

ERT is extremely effective for Pompe disease. However, its efficacy is decreased by antibodies against infused enzyme. We have previously shown that parenteral administration of an antibody against murine CD3 induced immune tolerance against the enzyme (Ohashi T, et al. Mol Ther, 2012). In a previous study we administered an anti-murine CD3 antibody in a murine model of Pompe disease. This antibody did not react with human CD3 antigens. To assess the possible clinical application of this strategy, we administered an anti-human CD3 antibody (otelixizumab) to wild-type mice expressing human CD3 by means of an infusion regimen similar to that used in the previous study. In this immunization protocol, parenteral administration of anti-human CD3 antibody also reduced the titer of antibodies against the enzyme.

Comparison of therapeutic effects of BMT, ERT, and combination therapy with ERT and BMT for LSDs

We have performed BMT, ERT, and combination therapy with both treatments in a murine model of mucopolysaccharidosis (MPS) type II and compared the efficacy of each treatment by means of an assay for tissue glycosaminoglycans (GAGs), which are storage materials in MPS II. The ERT reduced GAGs more profoundly than did BMT. Moreover, ERT had an additive effect to BMT. However, none of treatments reduced GAGs in the brain.

Disease modeling of late-onset Pompe disease-specific iPS cells

As in infantile Pompe disease, cardiovascular complications have been documented in late-onset Pompe disease. To clarify the mechanisms of cardiac involvement in late-onset Pompe disease, we have generated late-onset Pompe disease iPS cells and differentiated them into cardiomyocytes for disease modeling. Cardiomyocytes derived from

late-onset Pompe disease iPS cells exhibited disease-specific hallmarks, such as massive glycogen accumulation and lysosomal enlargement. Our results suggest that pathological changes in differentiated cardiomyocytes might explain the cardiovascular complications in late-onset Pompe disease.

Novel therapy for Pompe disease by using an enzyme-stabilizing agent

An ERT with recombinant human acid alpha-glucosidase (GAA) was recently approved for treating Pompe disease. This ERT prolongs survival and decreases cardiac muscle pathology, but has several problems, such as resistance in skeletal muscles and production of antibodies against recombinant human GAA. Therefore, an alternative method of addressing GAA deficiency is needed for the effective treatment of patients with Pompe disease. We have previously shown that the proteasome inhibitor bortezomib can exert a positive effect on mutant GAA in fibroblasts from a patient with Pompe disease carrying the specific mutation. However, bortezomib has not been fully characterized as an enzyme-enhancement molecule that is effective for multiple GAA mutations. In this study, we investigated the effect of bortezomib treatment on mutant GAAs in patient fibroblasts and transiently expressed cells. Bortezomib increased the maturation and residual activity of GAA in patient fibroblasts carrying the M519V and C647W mutations. Enhanced colocalization of GAA with lysosomal marker lysosome-associated membrane protein 2 was also observed in patient fibroblasts after treatment with bortezomib. When mutant GAAs were overexpressed in HEK293T cells, bortezomib increased the activity of M519V and C647W in these cells (by factors of 1.3 and 5.9, respectively). These results indicate that bortezomib enhances the activity of some GAA mutants, such as M519V and C647W.

Analysis of α-galactosidase A gene mutations from patients with Fabry disease by complementary DNA analysis and multiplex ligation-dependent probe amplification

Background: Fabry disease is characterized by the deficient activity of the enzyme α-galactosidase A (GLA). We revealed 4 new mutations of the *GLA* gene in 5 families using multiplex ligation-dependent probe amplification (MLPA) and *GLA* complementary (c) DNA analysis.

Results: Mutation analysis of *GLA* cDNA showed: 1) insertion of intron 3 (1 family), 2) insertion of intron 4 (2 families), 3) exon-skipping of exon 4 (1 family), and 4) exon-skipping of from exons 2 to 5 (1 family). Analysis of genomic DNA showed: 1) an IVS3+395 (G>C) point mutation, 2) an IVS4+330(113b) insertion mutation, 3) a L1 gene insertion mutation (exon 4 #745), and 4) a Del 5.5-kb (Int1-Ex5) deletion mutation.

Discussion: 1) The IVS3+395 (G>C) might induce alternative splicing. 2) This 113-base pair insertion might function as a splicing enhancer. 3) The exon-skipping by L1 has been reported in some genetic diseases. 4) An inverted repeat sequence is present within the deletion region of the 5′- and 3′-ends of DNA. This sequence might be involved in the deletion mechanism. The MLPA method and cDNA analysis method are useful for *GLA* gene mutation analysis, especially in large deletion/insertion mutations and functional gene mutations in introns.

Gene therapy using a lentiviral vector system and homologous recombination using zinc finger methods for LSDs

We are investigating the effects 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 treatment 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 including the iduronate sulfatase (IDS) gene. 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. 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 tried the zinc finger system for site-specific homologous recombination in the *GALC* gene *in vitro* and succeeded in exchanging a specific sequence of the *GALC* gene 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.

Antitumor effect and application to gene therapy of nafamostat mesilate for cancers of the digestive tract

Recent studies have demonstrated that nuclear factor (NF)-κB plays important roles in the regulation of cell apoptosis, inflammation, and oncogenesis. Inhibition of NF-κB is a potential new strategy for the treatment of cancers. We have previously reported that nafamostat mesilate, a serine-protease inhibitor, inhibits NF-κB 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-κB 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. Now we are investigating triple combination therapy with gemcitabine, nanoparticle albumin-bound paclitaxel, and nafamostat mesilate, which is the next standard therapy for pancreatic cancer. Recently we have investigated the antitumor efficacy of combination therapy with nafamostat mesilate and radiation for pancreatic cancer. In addition to inducing apoptosis, nafamostat mesilate was found to promote the efficacy of cell arrest at the G2/M checkpoint. Recent studies have found that human CD40 ligand (CD40L) gene delivery has a direct antitumor effect via CD40-CD40L interaction. However, CD40L enhances activation of NF-KB. We have previously reported that nafamosiat mesilate inhibits NF-KB activation and enhances apoptosis caused by adenovirus vector-mediated tumor necrosis factor α in pancreatic and hepatocellular carcinomas. Therefore, we have investigated the efficacy of combination therapy with nafamostat mesilate and adenovirus vector-mediated CD40L gene therapy for pancreatic cancer.

Publications

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Institute of DNA Medicine
Department of Oncology

Sadamu Homma, *Professor and Director*
Shigeo Koido, *Associate Professor*
Mikio Zeniya, *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

A phase I clinical study of immunotherapy against advanced pancreatic cancer using dendritic cells pulsed with WT1 class I and II peptides
Wilms' tumor 1 (WT1) is a tumor antigen recognized by specific T cells. Vaccination with dendritic cells (DCs) pulsed with WT1 class I and II peptides would stimulate WT1-specific cytotoxic T cells, as well as helper T cells, leading to the induction of potent WT1-specifc antitumor immunity. In 2013, 7 patients with advanced pancreatic cancer were treated with this DC therapy combined with gemcitabine. Four of these patients have been followed up in good condition as outpatients. All 4 patients showed positive skin reaction tests for WT1.

Immunotherapy against glioblastoma using a DC vaccine
Patients with glioblastoma treated with the combination of temozolomide and DC therapy have survived significantly longer than those treated with temozolomide alone. Analyses by tetramer assay indicated that 3 of 6 patients treated with both temozolomide and DC therapy showed induction of cytotoxic T cell responses specific for WT1, glycoprotein 100, and melanoma-associated antigen family A, 2.

Generation of artificial protein vaccine inducing potent cellular immunity
Artificial proteins composed of cytotoxic T-lymphocyte (CTL) epitopes, helper epitopes, and intercalated peptides of ovalbumin were generated using the MolCraft system for protein evolution. Uptake of the artificial protein via the scavenger receptor on antigen-presenting cells might be closely associated with the induction of cellular immunity. An artificial WT1 protein for use in cancer vaccines is now being generated on the basis of the specific protein structure inducing cellular immune responses.

Exploitation of antigenic peptides for T-cell responses generated from mutated gene products of cancer
Immunogenic proteins might be generated from gene mutations of cancer cells, and such

gene products might serve as tumor antigens for immunological tumor rejection. Structures of proteins generated by gene mutation were determined in human prostate and pancreatic cancer cells, and antigenic peptides for specific T-cell responses derived from the mutated proteins are now exploited by liquid chromatography/tandem mass spectrometry analysis.

Intensified antibody therapy by enhancement of target molecular expression

Antibody therapy targeting human epidermal growth factor receptor 2 (HER2) was not effective against pancreatic cancer because of low HER2 expression. We found that treatment with gemcitabine enhanced HER2 expression on pancreatic cancer cells. Gemcitabine-treated pancreatic cancer cells were more sensitive to trastuzumab-emtansine, a conjugate of an anti-HER2 monoclonal antibody and a chemotherapeutic agent, through gemcitabine-induced enhancement of HER2 expression. Gemcitabine also increased CD20 expression on human diffuse large B cell lymphoma cells. Complement-dependent cytotoxicity mediated by rituximab was enhanced by pretreatment of human diffuse large B cell lymphoma cells with gemcitabine.

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 CTLs and PD ligand (PD-L) 1 on cancer cells induces apoptosis of CTLs, PD-1/PD-L1 plays an important role in cancer-related immune suppression. The expression of PD-L1 on cancer cells is generally induced by interferon-gamma produced by CTLs attacking cancer cells. We found that treatment of cancer cells with nafamostat mesylate significantly suppressed interferon-gamma-induced PD-L1 expression. Nafamostat mesylate might contribute to the inhibition of cancer-related immune suppression and enhance the antitumor effect of anti-PD-L1 antibody therapy.

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Institute of DNA Medicine
Department of Molecular Genetics

Hisashi Yamada, *Professor and Director*

General Summary

The etiology of illness is based on the relationship between individual intrinsic factors and external environmental factors. Recently, the value of genetic factors has been assessed. The genetic involvement in disease occurs through DNA alignment disorders and epigenetic deregulation. The diseases we are interested in are cancers and neurodegenerative disorders. Many approaches have been taken to overcome these intractable diseases; we are now studying these diseases from the view-point of epigentical control.

Research Activities

Cancer Molecular Biology

In the past 3 decades, the genetic analysis of cancer cells has made remarkabl progress. Cancers develop when mutations accumulate in normal cells. Our previous research disclosed many mutations in each cancer by studying candidate genes, one at a time. However, the mutations of many candidate genes can be studied at one time with the high-throughput sequencer. By applying this method for a pediatric patient with double cancer, we could speculate that an affected stem cell was the cause of both cancers. These findings will help reveal the etiology of cancer and lead to new treatments.

Molecular pharmacology of anticancer agents

We are investigating the actions of bromodomain and extraterminal domain inhibitors (I-BET151 and JQ1). Bromodomain-containing proteins attach transcription-related proteins to acetylated histones. This megaprotein complex forms a set of active transcriptional machinery that regulates genes for cell proliferation and survival. However, the sensitivity of leukemia cells to I-BET151 differs markedly among cell lines. The reason, why only a few cancers are sensitive for 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.

Molecular genetical approach to neurological diseases

The homozygous deletion and mutation of the survival motor neuron (*SMN*) 1 gene causes the hereditary neurodegenerative disorder, Spinal Muscular Atrophy (SMA), which is characterized by progressive loss of alpha-motor neurons in the spinal cord. Initially we found that the heterogeneous nuclear ribonucleoprotein (hnRNP)

A2-specific knock-down repressed SMN synthesis (but increasing full-length transcripts of SMN2 gene) rather than increasing SMN by knock-down for hnRNP A1 (also increasing full-length transcripts by enhancing exon 7 splicing). The aim of this study was to characterize the molecular mechanism of hnRNP A2 specific RNA interference-mediated SMN reduction.

With the reverse transcriptase-polymerase chain reaction (RT-PCR) and the pulse-labeling and analysis of newly synthesized RNA and proteins, we found that this regulation was controlled at the translation level. Sucrose-gradient ultracentrifuge studies showed that SMN mRNA interacted less with polyribosomes after hnRNP A2 depletion. Pull-down analysis of RNA-protein complexes by RT-PCR, matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) and immunoblots analyses showed that hnRNP A2 directly interacted with SMN mRNA and with other RNA binding proteins, hnRNP C1/2 and M dependent on RNA binding. The RNA pull-down assay revealed the interaction between hnRNP A2 and SMN mRNA via a UUUAGG A2 binding consensus motif at 3′-UTR. Our findings define a new regulatory mechanism for controlling SMN protein production provide new insight into cellular hnRNP A2 function in translation, and suggest new avenues for developing drugs to treat SMA.

Alzheimer disease is a progressive and incurable degenerative condition. We 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 Alzheimer disease. We are now studying the epigenetic regulation of these 2 genes.

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Institute of DNA Medicine
Department 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

A single dose of interleukin 31 causes continuous itch-associated scratching behaviour in mice

Interleukin (IL) 31 is a T-cell-derived cytokine that induces severe pruritus, hair loss, and dermatitis and is involved in allergic diseases, such as atopic dermatitis and bronchitis. We investigated the effects of a single dose of mouse IL-31 on scratching behavior in comparison with scratching behavior induced by spontaneous skin lesions or serotonin (5-HT) in NC/Nga and BALB/c mice. About 3 hours after administration, intradermal injection of IL-31 caused a gradual increase in long-lasting scratching (LLS, more than 1.5 seconds), which was followed by a gradual decrease over 24 hours after administration. Intradermal injection of IL-31 significantly increased the total LLS counts/24 hours but did not increase short-lasting scratching (SLS, 0.3-1.5 seconds). In NC/Nga mice with skin lesions, the LLS counts, but not the SLS counts, were significantly higher than those in NC/Nga mice without skin lesions. We also investigated 5-HT-induced scratching in BALB/c mice; we found that SLS counts, but not LLS counts, increased immediately after the injection and then decreased to baseline after 20 minutes. These results suggest that IL-31 participates in the sensation of itching and promotes scratching behavior in NC/Nga mice with skin lesions, an animal model of atopic dermatitis.

Cross-reactivity at the T-cell and B-cell levels within the Cupressaceae family and Taxoidiaceae subfamily in patients with Japanese cedar pollinosis

Allergens of the Cupressaceae family and Taxoidiaceae subfamily are a major cause of pollinosis in several geographic areas. A comparison of immunoglobulin E (IgE) epitope regions has shown that allergens from several taxa cross-react. However, the cross-reactivity at the T-cell level within the Cupressaceae family and the Taxoidiaceae subfamily has not been analyzed in detail.

We evaluated the cross-reactivity at the T-cell and B-cell levels between Cupressaceae and Taxodiaceae allergens in patients with Japanese cedar pollinosis. Crude extracts of Cupressaceae and Taxoidiaceae pollens were used as allergens for analysis. Proliferative responses to the allergens of peripheral blood mononuclear cells from patients were

examined for cross-reactivity at the T-cell level, and measurement of CD203c expression on basophils was used to examine allergenic cross-reactivity at the B-cell level. Cry j 1-specific T-cell lines were also used to analyze cross-reactivity at the T-cell level among these families.

Patients with Japanese cedar pollinosis were divided into 2 groups: those who showed reactions to all allergens tested within the Cupressaceae family and Taxoidiaceae subfamily and those who showed reactions to some allergens at the T-cell level. The existence of a common T-cell epitope among the Cupressaceae family and the Taxoidiaceae subfamily, which was a major human T-cell epitope in Cry j 1 in patients with Japanese cedar pollinosis, was identified with a Cry j 1 p211-225-specific T-cell line. Even if the cross-reactivity to each allergen was not found at the T-cell level, serum IgE antibodies showed cross-reactivity to all allergens tested within the Cupressaceae family and Taxoidiaceae subfamily in patients with Japanese cedar pollinosis.

A common T-cell epitope in group 1 allergens among the Cupressaceae family and Taxoidiaceae subfamily was identified for the first time. The cross-reactivity at the T-cell level between Cupressaceae and Taxoidiaceae species was determined depending on whether T-cells of a patient could recognize peptides of the common T-cell epitope, which was restricted by the HLA haplotype of the patient. On the other hand, serum IgE antibodies of the patients showed cross-reactivity to all allergens of Cupressaceae family and the Taxoidiaceae subfamily, because the group 1 or 2 allergens are structurally similar among the Cupressaceae family and the Taxoidiaceae subfamily.

These results suggest that symptoms in patients with Japanese cedar pollinosis develop at the T- or B-cell level in response to allergens of the Cupressaceae family and the Taxoidiaceae subfamily.

Enhancement of cytotoxic T-lymphocyte induction with cationic liposome by the modification of N-glycan structure

We have developed an adjuvant inducing cytotoxic T lymphocytes (CTLs) via cross-presentation with a cationic liposome. After ovalbumin (OVA) protein and the adjuvant were mixed and administered to mice, the CTLs against the OVA epitope were induced within 5 days independently of helper T cells. Also, CTL-inducing activity was not decreased in TLR-2/4/9 knockout mice.

With this adjuvant, we compared the CTL-inducing activity of OVA derivatives with different N-glycan structures. Mice were immunized with this adjuvant mixed with chicken OVA, OVA expressed in Escherichia coli, or OVA expressed in HEK-293F cells, and then the activity of CTL induction was analyzed.

All antigens had the ability to induce CTLs, but the activities were different. It is well known that, the structures of N-glycan differed among secreted and unsecreted proteins when secretory proteinswere expressed in HEK-293 cells. Hence, the unsecreted proteins were highly mannosylated, and we reproduced the N-glycan structure of OVA with an inhibitor of N-glycan modification. The secretion of modified OVA was not inhibited, but the ability to induce CTLs with a cationic liposome was enhanced through the modification of N-glycan.

Because the modified OVA without adjuvant failed to induce CTLs, comparing the effects

of N-glycan modification on the basis of cell-membrane receptors is difficult. However, after uptake of OVA by antigen-presenting cells, there may be some mechanism to induce cross-presentation with cationic adjuvants inside cells.

Publications

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Institute of DNA Medicine
Department of Molecular Cell Biology

Yoshinobu Manome, *Professor*

General Summary

Our department uses molecular approaches to analyze physiological and pathological cellular events. Molecular cell biology techniques are practical methods for assessing cellular events. Our methods include modification of nucleic acid transcription and expression by transfection of DNA or short interfering RNA. Also, labeling of molecules with fluorescent nanoparticles, conjugation to sensors, and amplification with radiolabelled materials are our specialties. By introducing these techniques, we are helping to solve clinical problems.

Research Activities

Application of therapeutic ultrasound for delivering nucleic acids to malignant glioma cells

Glioma is an intractable disease of the central nervous system. Because the prognosis after surgical removal remains poor, alternative therapies, such as chemotherapy, radiotherapy, and immunotherapy, have been developed. Despite the poor prognosis, metastasis outside the central nervous system is rare and the cause of death in most cases is local recurrence. Thus, long-term survival might be possible if a more effective local therapy were developed. We have developed such a therapy using therapeutic ultrasound irradiation and have reported the beneficial effect of therapeutic insonation in combination with microbubbles. In the present study, to enhance the therapeutic efficacy, an additional nucleic acid delivery system was developed. This year, we accomplished the experimental production of the device.

Transcription of urocortin and corticotropin-releasing factors in gastric carcinoma cells

Urocortin (Ucn) and corticotropin-releasing factors (CRFs) and their receptors are expressed in many organs, including those of the central nervous system. Previously, we demonstrated the expression of messenger (m) RNAs of Ucn I, II, III, and CRF and CRF receptors (CRFR) 1 and 2 in malignant glioma cell lines. This year, we examined transcription in the STKM gastric carcinoma cell line. This cell line expressed mRNAs of UCN 1 and 2 and CRFR2. However, unlike malignant glioma cells, they did not express the mRNAs of UCN3 and CRFR1. The transcription pattern was not affected by growth or cytotoxic signals.

Peptide-tracking of Ucn I in human glioblastoma cells using the Ucn I-fluorescent protein hybrid protein

Due to the lack of knowledge about the secretion mechanism of Ucn I, we investigated

the secretory pathway of Ucn I using human glioblasotoma cells (A172 and U138-MG cells) and the Ucn I-fluorescent protein hybrid protein-expressing plasmid. Immunocytochemical studies revealed immunoreactivity for Ucn I, CRFR1, and CRFR2 in human gliblastoma cells, but the expression patterns of immunoreactivity were different. Next, a Ucn I-fluorescent protein hybrid protein-expressing plasmid was constructed and introduced into A172 human glioblastoma cells, and the intracellular dynamics of Ucn I was tracked with fluorescent microscopy. Fluorescent microscopy suggested that Ucn I is secreted via the constitutive pathway. This result also suggests that the secretion of Ucn I is correlated with the level of mRNA production.

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

Recent technical innovations have enabled mass production of various nano materials. Although nano materials are used for foods and cosmetics owing to their improving quality, their safety is still under investigation. Recently, several studies have shown that nano materials (< 100 nm) penetrate brain tissue. However, whether the penetrating nanoparticles affect neuronal activity is unknown. Direct *in-vivo* assay to reveal the toxic neuronal effects and mechanisms of nanoparticles may be difficult, because most particles are distributed to other organs, and penetrating particles localize in small areas of the brain. Therefore, to understand the toxic effects of nanoparticles we have developed an *in-vitro* brain model for use with cellular assays. Our bottom-up model includes 1) a blood-brain barrier model, which indicates the apparent permeability coefficient, and 2) cellular assays. In our concept, nanoparticles that were able to penetrate were selected by means of the blood-brain barrier model, and then possible toxic effects and mechanisms were assessed with integration of the cellular toxicological results using the penetrating particles. With this novel model, we examined the toxic effects of nanoparticles and microparticles on human neural stem cells. We found that 30-nm silica nanoparticles at high concentration (0.1 mg/mL) affected the viability and differentiation of human neural stem cells. This concentration may be helpful for considering the maximal dose for *in-vivo* administration.

Publications

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Institute of DNA Medicine
Project Laboratory for Kidney Regeneration

Takashi Yokoo, *Professor and Director*

General Summary

The critical shortage of organs has driven new technologies, such as tissue engineering and regenerative medicine, to achieve functional kidney replacement. Our previous studies have shown that a xenobiotic developmental process for growing xenoembryos allows exogenous human mesenchymal stem cells (MSCs) to undergo epithelial conversion and form a nephron that produces urine and erythropoietin. These findings suggest that MSCs can be a source of cells for future renal regeneration. Furthermore, MSCs are easy to obtain in large numbers and are not costly to establish.
Previously, we used bone marrow-derived MSCs from healthy volunteers, although whether these MSCs differ from MSCs from patients undergoing dialysis is unclear. This uncertainty is due to patients with end-stage renal failure having been exposed over long periods to uremic toxins, which may affect the viability and regenerative capacity of their MSCs and make them unsuitable for kidney regeneration. This year, we have demonstrated differences in the gene and protein expression of MSCs from patients with end-stage kidney disease (ESKD) and healthy individuals using a polymerase chain reaction array and Western blot analysis. We found that long-term uremic conditions lead to persistent and systematic downregulation of *in-vitro* gene and protein expression of P300/cAMP response element-binding protein (CREB)-binding protein (CBP)-associated factor (PCAF) and low angiogenesis activation of *in vivo* assay in the MSCs of patients with ESKD. Furthermore, we demonstrated that the hypoxic responses of PCAF, hypoxia-inducible factor-1α (HIF-1α), and vascular endothelial growth factor (VEGF) were significantly blunted in MSCs from patients with ESKD. We propose that the transcriptional regulation by low levels of PCAF is inappropriately controlled by environmental factors representing long-term ESKD. Low expression of PCAF induced by long-term ESKD may lead to down-regulation under hypoxia of HIF-1α and VEGF in MSCs from patients with ESKD undergoing long-term dialysis (KD-MSCs). These findings should help to elucidate the mechanisms of the effects of uremic toxins. Further studies are needed to clarify the relationship of CKD and the down-regulation of PCAF.

Research Activities

We have previously demonstrated that MSCs differentiate into functional kidney cells capable of urine and erythropoietin production and can, therefore, be used for kidney regeneration. However, the viability of MSCs from patients undergoing dialysis might be affected under uremic conditions. In this study, we isolated KD-MSCs (mean duration of dialysis: 72.3 months) and MSCs from healthy control subjects (HC-MSCs) to compare their viability. The KD-MSCs and HC-MSCs were assessed for their prolifera-

tion potential, senescence, and capacity to differentiate into adipocytes, osteoblasts, and chondrocytes. Gene expression of stem cell-specific transcription factors was analyzed with polymerase chain reaction array and confirmed with Western blot analysis at the protein level. No significant differences in proliferation potential, senescence, or differentiation capacity were observed between KD-MSCs and HC-MSCs. However, gene and protein expression of PCAF was significantly suppressed in KD-MSCs. Because PCAF is a histone acetyltransferase that mediates regulation of HIF-1α, we examined the hypoxic response in MSCs. The HC-MSCs but not KD-MSCs showed up-regulation of PCAF protein expression under hypoxia. Similarly, expression of HIF-1α and VEGF did not increase under hypoxia in KD-MSCs but did increase in HC-MSCs. Additionally, a direct *in vivo* angiogenesis assay showed decreased angiogenesis in KD-MSCs. In conclusion, long-term uremia leads to persistent and systematic down-regulation of *PCAF* gene and PCAF protein expression and low angiogenesis activation in MSCs from patients with ESKD. Furthermore, expression of PCAF, HIF-1α, and VEGF are not up-regulated by hypoxic stimulation of KD-MSCs. These results suggest that the hypoxic response may be blunted in MSCs from patients with ESKD.

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Department of Neuroscience
Laboratory of Neurophysiology

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 (CNS) underlying various specific functions, such as autonomic regulation and pain sensation. In particular, plastic changes of the CNS "wiring" realized through the variability of synaptic connections in response to various environmental changes form the core mechanism for optimizing human and animal behaviors. We use approaches at the molecular, cellular, and network levels, including the patch-clamp recording of synaptic currents, the real-time imaging of the intracellular Ca^{2+} concentration, and optogenetic approaches to activate a specific set of neurons by light, in living brain tissues from normal animals, animal models of various diseases, and animals subjected to experimental manipulation of gene expression and combine them with the 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 unequivocally demonstrated monosynaptic connections between these nuclei using optogenetics with channelrhodopsin-expression systems and measurement of light-evoked postsynaptic responses. These findings further confirm the notion that the chronification process of pain involves potentiated links 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 cue and an 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. This finding is the first to demonstrate the role of a nonthalamic nocicep-

tive pathway in fear learning.

Glia–neuron interaction at central synapses

To clarify the role played by the transfer of lactate from astrocytes to neurons in synaptic transmission, we analyzed the effects of selective inhibitors of monocarboxylate transporters on synaptic transmission in neurons of the nucleus of the solitary tract, the lateral amygdala, cerebellum, hippocampus, and the hypoglossal nucleus. We found that lactate transport is essential for maintaining the postsynaptic responses both in the presence and the absence of glucose supply in most of the brain synapses.

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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 using high-performance computers and medical virtual reality systems has expanded the possibilities for diagnosis, treatment, surgery, and medical education. The Institute for High Dimensional Medical Imaging has, therefore, established a system that facilitates cooperative research and development with international researchers and organizations.

Research Activities

Clinical application of high-definition, real-time medical imaging
We are performing research on the development of medical high-definition imaging technology and its clinical application using functional and morphological data obtained with X-ray computed tomography (CT) and magnetic resonance imaging.
We are developing a 4D motion system for analyzing human activities, such as the motions of the whole body. The system is driven by motion data obtained from anatomical and skeletal muscle models reconstructed from X-ray CT data sets.
This year we developed a gait-analysis system to simulate the changes to the knee adduction moment in varus-valgus deformity of the knee during gait and performed validations using preoperative and postoperative gait data from a patient with osteoarthritis of the knee.
This research is being performed by departments in our university in collaboration with Osaka 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 (NOTES). Robotic instruments enter the abdominal cavity orally and are used to perform surgery on the abdominal organs.
This year we developed an "over tube system" that can bend and maintain the posture of the robot in the abdominal cavity. 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.

This year we attempted to combine the surgical simulator and the navigation system. To share the surgical plan among surgery staff during the operation, the plan data made by preoperative surgical simulation transferred to the navigation system and displayed on navigation screen.

Development of an image-guided surgery system

We are developing a system that can display blood vessels and tumors at the back of the surgical field in the form of 3D geometric models in multiple layers on the surgical field screen. Such improvements will make the navigation system more intuitive.

This year the Department of Surgery and the Department of Otorhinolaryngology again jointly performed navigation surgery in the high-tech navigation operating room of Daisan Hospital as a semiroutine procedure. From this joint research, we developed a pointing device suited for various surgeries and a registration method with improved accuracy which was applied to clinical examination. We also started to develop a navigation system for laparoscopic surgery to deal with new surgical procedures.

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. In addition we developed methods to display in 3D, wounds of victims in incidents where there are only X-ray CT data in films or photographs from forensic autopsy.

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

Publications

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Institute of Clinical Medicine and Research

Toya Ohashi, *Professor and Director*
Yoshihisa Namiki, *Associate Professor*
Akihito Tsubota, *Professor*

General Summary

The aim of our research is to fill the gap between clinical medicine and basic medicine. We have made good progress in the development of a drug delivery system using nanotechnology. In addition, this year we developed methods to eliminate radioactive compounds using magnetic basket-shaped nanosized capsules containing decontaminants. We also made progress in gene technology, especially in the treatment of hepatitis C virus (HCV) infection and liver cancer. Other major research topics are a transporter of ribavirin into hepatocytes and the function of microRNA/messenger (m) RNA. In the field of lipid metabolism related to atherosclerosis, we have reassumed lipoprotein cholesterols separated using our newly developed ion-exchange chromatography; last year we used this chromatography method to measure lipoprotein (a), atherosclerotic lipoproteins with a special apolipoprotein called apolipoprotein (a).

Research Activities

Transporter gene in the treatment of chronic HCV infection
Ribavirin is the main component of the combination treatment for chronic HCV infection, even though great progress has been made in developing direct-acting antiviral agents against HCV. In ribavirin-combined treatment, exposure of HCV in hepatocytes to ribavirin is critical for virus eradication. 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.

Comprehensive gene expression profiling analysis of microRNA/mRNA in liver tissue
We are profiling and analyzing the expression of microRNA/mRNA in the liver tissue of patients with chronic HCV infection who would receive pegylated interferon-alpha plus ribavirin combination treatment. We have analyzed whether the microRNA/mRNA candidates can be associated with treatment response in chronic HCV infection. We have found the novel interaction between microRNA and mRNA in the replication and lifecycle of HCV. Currently, the functions of microRNA/mRNA are being investigated in detail.

The fabrication of "3D organic/inorganic-hybrid structure" as a future theranostic (therapy + diagnostic) and preventive nanomedicine
(Funding Program for Next-Generation World-Leading Researchers [JSPS])
(Funding for the Development of Decontamination Technology [Ministry of the Environ-

ment: Government of Japan])
Free manipulation of the movement of drugs with remote-controlled light/magnetism/ultrasound used in cutting-edge medical technology is expected to be a next-generation technology. Remotely manipulating the speed and position of nanoparticles, which are mineral capsules that respond to various types of physical energy and are filled with organic drugs, will lead to an innovative technology that allows "pinpoint" prevention, diagnosis, and treatment.
We aim to realize innovative nanomedicine in which we can remotely control the accumulation, release, and effects of drugs with nanosized capsules that efficiently convert light, magnetic, and ultrasonic energies. This is unprecedented research in which we can apply Japan's world-leading nanotechnology to medicine. It will allow highly sensitive, rapid diagnosis and highly effective treatment that is gentle to the body for incurable diseases and for diseases that are difficult to diagnose. The realization of medical care that is gentle to the weak, such as elderly persons, will help promote a long and healthy life, reduce healthcare costs, and lead to the development of the healthcare industry. Moreover, because this technology can precisely control the behavior of drugs, it can be applied to diverse areas, such as pharmacology, biotechnology, agriculture, and environmental science.

Studies of lipid metabolism and atherosclerosis
The relationship between diet and the incidence of cardiovascular disease among Japanese was investigated exhaustively through large-scale cohort studies in Japan, and their results were published in the *Journal of Atherosclerosis and Thrombosis*. Effects of carbohydrate co-feeding with lipids on postprandial hyperlipidemia were investigated with the measurement of serum levels of apolipoprotein B48. An incubation study using bacteriophages was performed to examine the antiviral effects of plasma fractions, and the antiviral fraction was extracted from human plasma. We developed a new high-performance liquid chromatography (HPLC) method for measuring lipoprotein (a) (published in the *Journal of Lipid Research*). By measuring very low density lipoprotein cholesterol with this HPLC, we proved the benefit of therapeutic exercise for reducing remnant lipoproteins. The effects of carbohydrate co-feeding with lipids on postprandial hyperlipemia, with measurement of serum levels of apolipoprotein B48, in healthy Japanese subjects were investigated, and the results were reported at the scientific meeting of the International Symposium on Atherosclerosis.

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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 impact on future opportunities and progress in biomedical research.

Research Activities

Disease modeling with iPS cells
We have generated 2 lines of iPS cells derived from patients with metachromatic leukodystrophy, a lysosomal storage disease. Furthermore, we have started to generate iPS cells from patients with familial Parkinson's disease.

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. 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 rescued 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.

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 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. We investigated the causal role of the gene mutation in the ALS/FTLD phenotypes using gene knock-in mice. Two types of mutant TDP-43 knock-in (mTDP-43 KI) mice with mutations in different sites of the gene were generated to investigate the biological effects of each mutation. Considerable differences in phenotypes were observed among mTDP-43 KI mice and wild-type mice: poor weight gain and loss of spinal motor neurons, which is related to ALS symptoms, and a decrease in anxiety levels, which may be related to FTLD. Interestingly, one type of knock-in mouse exhibited predominantly motor dysfunction, and the other type showed behavioral abnormalities. These observations indicate that mutation sites of the *TDP-43* gene are the predominant factor which sink into multimodal or exclusive pathologies. Our results, referencing *TDP-43* mutations, will provide new insights into the pathophysiology of 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 PARK1- and 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. Advances in disease modeling using genetically modified primates will have a huge impact on future opportunities and progress in the research on neurodegenerative diseases.

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Medical Engineering Laboratory

Masayuki Yokoyama, *Associate Professor and Director* Kouichi Shiraishi, *Assistant Professor*

General Summary

The Medical Engineering Laboratory provides new and essential techniques for developments of medicine. There are 2 key technologies in our laboratory: ultrasound and polymer nanoparticles. 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 departments, both in our university and hospitals and others. For the other key technology, polymer nanoparticles, we have applied polymeric micelles to drug delivery systems (DDSs). Recently, we have also applied a polymeric micelle carrier system to a magnetic resonance imaging (MRI) contrast agent. In particular, we have studied the polymeric micelle MRI contrast agent for the diagnosis of acute ischemic stroke. The polymeric micelle carrier system has become a great system for diagnosing and treating ischemic stroke.

Research Activities

Medical application of ultrasound

We have applied transcranial ultrasound for the sonothrombolysis of acute ischemic stroke. For this condition, injection of tissue plasminogen activator (t-PA) within 4.5 hours of onset is the only effective thrombolytic therapy. Therefore, safe and effective technology to enhance the therapeutic effects of t-PA would be highly beneficial. We have shown that transcranial ultrasound for sonothrombolysis can enhance the thrombolytic activity of t-PA and increase the recanalization rate. However, although the recanalization rate is increased, other groups have shown that standing waves at an ultrasound frequency of 300 kHz is associated with a high risk of brain hemorrhage. Therefore, we have applied ultrasound at a medium frequency, 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 a medium frequency. We have found that our modulation method, which involves periodic selection of random ultrasound frequencies, 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. We have also developed a novel medical device that can detect blood clots in the carotid artery.

Polymeric micelle drug carrier systems

Self-assemblies of synthetic polymers, polymeric micelles, have been actively developed for drug targeting. Yokoyama, the director of this laboratory, is an international pioneer in the development of polymeric micelle targeting systems. Currently, 4 formulations of

polymeric micelle anticancer drugs are undergoing clinical trials in Japan, Europe, and the United States. We are trying to establish 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 diseases. We have shown that this MRI contrast agent has the ability to target solid tumor sites and exhibits high signal intensity in extremely small solid tumors. We have been studying a novel application of the MRI contrast agent for brain ischemic stroke. In a 3-hour middle cerebral artery occlusion (MCAO) model, the MRI contrast agent quickly showed high signal intensity within part of the ischemic hemisphere. The high signal intensity area did not appear on diffusion-weighted images or T2-weighted images. Furthermore, the image obtained with the MRI contrast agent was not obtained with a conventional low-molecular-weight MRI contrast agent. These findings indicate that the MRI contrast agent we developed might be used to assess the hemorrhage risk of ischemic stroke. The MRI contrast agent must be further optimized to be suitable of this purpose. Therefore, the polymeric micelle carrier system will be useful in both the diagnosis and treatment of brain ischemic stroke. This is our new and valuable challenge.

We have been studying polymeric micelle-related immune responses. The phenomenon exhibits specificity for polyethylene glycol (PEG), which is used for block copolymer. The PEG-specific antibody (anti-PEG immunoglobulin M) is generated when either polymeric 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 polymeric micelles and PEGylated liposomes possess PEG and generate anti-PEG antibodies, the polymeric micelles exhibited little or no change in behavior after priming. We further evaluated the effect of the anti-PEG antibody on the behaviors of both polymeric micelles and PEGylated liposomes. We found that both carriers generated nearly the same numbers of antibodies; however, although the injected dose includes nearly the same number of PEG chains, polymeric micelles were 10 times as numerous as PEGylated liposomes. Five to 10 anti-PEG antibodies can bind to a 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 through the use of synchrotron radiation (at the Super Photon Ring 8 Gigaelectronvolt facility, Hyogo Prefecture, Japan). The precise measurement accurately detects the inner core structure of polymeric micelles, and we have shown a correlation between the characteristics of polymeric micelles and their biological behavior, in particular, their pharmacokinetic behavior.

Publications

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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 antihyperlipidemic 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 submitted.

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.

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)—6 nurses and 1 pharmacist—now facilitate clinical trials. The CRCs have started to help with both registration trials and investigator-initiated trials. CRCs have been introduced into all registration trials since 2004; the quality and speed of these trials were much improved.

The Ministry of Health, Labour and Welfare started a New 5 Yearly Clinical Trial Action Plan to help registration trials to cope with trials performed abroad. This action plan selects 10 core hospitals and 30 major clinical trial institutions. The Jikei University Hospital was named a major clinical trial institution. According to this plan, we reinforced CRCs and introduced a data manager to improve the clinical trial system. We also introduced an information technology system for processing registration trial management.

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 others 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 on global health
4. Randomized trial to prevent food allergy

Publications

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Division of Clinical Epidemiology

Masato Matsushima, *Professor and Director*

General Summary

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

The research topics of our division include medical communications, assessment of the quality of medical care, behavioral medicine, outcome research, qualitative research, and disease-oriented epidemiological research. In particular, we aim to produce evidence in the field of primary care, which, despite being a front line of practice, suffers from a shortage of evidence.

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

Our postgraduate education concentrates on methods of clinical/epidemiological research and biostatistics. "The Educational Program for Primary Care on Clinical Research Methodology," which was started in 2007 with the financial support of the Ministry of Health, Labour and Welfare of Japan, was renewed as "Jikei Clinical Research Program for Primary-care" in 2009. The aim of this program is to teach primary-care physicians to be clinician researchers.

Research Activities

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

Although little is known about the prognosis of patients receiving home medical care in Japan, few prospective cohort studies involving elderly persons receiving home medical care have been performed in Japan. The EMPOWER-JAPAN study was started as a multicenter prospective cohort study to investigate in-home mortality and to 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 the Japan Society for the Promotion of Science.

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

The chronic care model was developed during 1990s in the United States to improve the care of chronic illnesses by refining the care-provider system, especially in a primary-care setting. The research plan consists of 2 steps. The first is to make an official Japa-

nese version of the assessment form "Assessment of Chronic Illness Care (ACIC)" by following the World Health Organization procedure, for example, translation, back translation. This step was finished. The second step is to compare the quality of diabetes care between specialists in diabetes and primary-care physicians as non-specialists.

Recognition and intention of gastrostomy and ventilator use in the care of older patients with advanced dementia: Differences among laypersons and healthcare professionals in Japan

In this cross-sectional study, a comparison was made between laypersons and healthcare professionals regarding the recognition and intention towards terminal care, such as gastrostomy and use of a ventilator.

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

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

Publications

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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 2013, about 450 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 African animal 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.

Development of a novel method of fecal occult blood testing in dogs and the effects of gastrointestinal parasitic infections on fecal occult blood levels in dogs
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 a small amount of blood in feces which is undetectable with the naked eye or under a microscope. The FOBT was originally developed as a screening test for alimentary canal tumors in human patients. However, the FOBT remains rarely used in veterinary medicine. In addition, little is known about its clinical significance, because the chemical FOBT is based on the peroxidase activity of hemoglobin. Thus, this chemical test has low sensitivity and specificity, because it often obtained false-positive or false-negative results if patients' diets contain hemoglobin of other species, myoglobin, or ascorbic acid (vitamin C). Therefore, a test subject must be placed on a restricted diet before a chemical FOBT. In particular, performing the FOBT for dogs and cats, which are the most common animals brought to small-animal clinics, is difficult because of their feeding habits and their various breeding

environments. We developed a FOBT for dogs, investigated its performance, and studied its indications. We demonstrated that our FOBT method is independent of a dog's diet, which might include the meat or blood of animals of other species or oranges, which contain vitamin C. In addition, we found that infection with a certain type of gastrointestinal parasite causes a significant increase of FOBT values in dogs. This increase was significantly decreased with antihelminthic treatment. This result suggests that our FOBT method is useful for screening for parasitic infections in human and animals in developing countries where these zoonotic parasitoses are common. We are now analyzing cases of gastrointestinal cancer in dogs.

Publications

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Radioisotope Research Facility

Kunihiko Fukuda, *Professor and Director* Yukio Yoshizawa, *Assistant Professor*

General Summary

The Radioisotope Research Facility was established to support medical and biological research with radioisotopes. The Facility also accepts the nonradioisotopic research that uses animals or recombinant DNA techniques. We have supported researchers by suggesting methods and practical techniques for experiments. Lectures and training courses were held for researchers and for medical students and graduate students. In 2013, 33 researchers from 11 departments and 18 students of 2 curriculums used the laboratory in this facility. Major nuclides used for experiments were ^{32}P, ^{51}Cr, ^{125}I, ^{14}C, and ^{3}H.
The Fukushima Dai-ichi nuclear power plant was damaged by the Tohoku-Pacific Ocean Earthquake and Tsunami on March 11, 2011. Large amounts of fission products and activation products were released into the environment by the accident. We focus on the study of the behavior and distribution of the radioactive materials in the environment as well as on radiation biology. Education related to radiation is also an interest.

Research Activities

Analysis of resistance mechanisms in radiation-resistant organisms
Tardigrades are small water-dwelling animals less than 1 mm in length. They are commonly called water bears because they walk slowly with 8 legs. They have adapted to growth at low temperatures and are found in Antarctica and the Himalayas, but they also inhabit moss in cities. Water bears can tolerate extreme environments, including ionizing radiation. To clarify the radiation-resistant mechanism of the water bear by transcriptome analysis and whole genome DNA sequencing, activated sludge was obtained from Ariake Water Reclamation Center, Bureau of Sewerage, Tokyo Metropolitan Government, to isolate the sludge water bear *Isohypsibius*. Nucleic acids were isolated from *Isohypsibius* and analyzed with a next-generation DNA sequencer. The results showed that samples from the sludge water bear were heavily contaminated with the nucleic acids of eukaryotic microbes. Therefore, we changed the sample with DNA of *Milnesium tardigradum* isolated from the moss *Brachymenium exile*. However, isolating enough *M. tardigradum* specimens for DNA preparation was difficult. The DNA prepared from *M. tardigradum* was amplified with the whole-genome amplification method. From 2 separate preparations, a total of 0.3 μg of DNA was obtained with multiple annealing and looping-based amplification cycles. The DNA size was 1 to 1.5 kbp, which is compatible with the next-generation sequence analysis.

Radioactive fallout in the environment
Radioactive materials from the accident at the Fukushima Dai-ichi nuclear power plant spread as far as the Kanto area. Soil and plant samples were examined with radiation

images using an imaging plate system. Imaging data of a bamboo shoot in June 2011 collected from Kawamata-cho, Fukushima Prefecture, suggest that its contamination by ^{134}Cs and ^{137}Cs was caused not by extraneous attachment but by absorption through roots or translocation from leaves. Radioactive cesium taken up into the plant body was present in high concentrations in the edible parts of the bamboo shoot, especially at the tip. We collected samples from the same bamboo grove for 3 years to obtain more information on annual trends and the circulation of radioactive cesium in the bamboo forest. The concentration of ^{137}Cs was measured as 2,600 Bq/kg in 2011, 600 to 900 Bq/kg in 2012, and 190 Bq/kg in 2013. Therefore, the amount of new radiocesium uptake is very small. Radiocesium was quantitatively measured in seawater collected from the Fukushima coast. The surface seawater was sampled about 1.5 km off the coast of the Fukushima Daiichi nuclear power plant in November 2013. Radioactive cesium was measured with the ammonium molybdophosphate method and gamma-ray spectrometry using a Ge-detector. The ^{137}Cs concentration was 0.02 to 0.08 Bq/L, and the ^{134}Cs concentration was 0.01 to 0.04 Bq/L. The ^{137}Cs concentration has not returned to the level of 0.001 Bq/L before the accident but has been significantly decreased from the level of several thousand Bq/L immediately after the accident, and the risk of migration to marine organisms from seawater need not be considered.

Study of radon

Radon is produced from the natural radioactive decay of uranium that exists in rocks and soil. Airborne radon activity is the second most common cause of lung cancer after smoking. However, in Japan, radon thermae or hot springs are popular for those who expect radiation hormesis. Radon is a chemically inert gas that can escape easily from the bath water into the air. The gas tends to concentrate in enclosed spaces, such as bathrooms. To assess the radon concentration of hot springs, the radon components must be measured repeatedly because it is affected by several factors, such as the uranium concentration in the basement, the underground structure, and atmospheric pressure. Samples were collected from 2 famous radon hot springs: Misasa Onsen (Misasa, Tohaku-gun, Tottori Prefecture) and Sarugajo Onsen (Tarumizu, Kagoshima Prefecture). The radon concentrations of the hot springs, measured with a liquid scintillation counter, were 1,070 Bq/L and 1,470 Bq/L, respectively. The radon concentration of air in these hot springs may exceed 100 Bq/m^3, which is the reference level proposed by the World Health Organization because the estimated transfer coefficient of radon between water and air is 1.0×10^{-4}.

Core Research Facilities

Yoshinobu Manome, *Professor*
Toshiaki Tachibana, *Associate Professor*
Takeo Iwamoto, *Associate Professor*

General Summary

The Core Research Facilities were reorganized on April 1, 2009, as the Research Center for Medical Sciences and consists of the Division of Fine Morphology, the Division of Biochemistry, and the Division of Advanced-Research Laboratory. The mission of the facilities is the facilitation of research in the university. Two systems are constituted for the use of the facilities

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

Monoclonal antibody to thyroid papillary carcinoma for patient screening

A monoclonal antibody against thyroid papillary carcinoma has been used to detect antigens in the membrane fraction of tumor cells or secreted by tumors in tumor-bearing patients. With the conjugation of the antibody, we developed a measuring method with the sandwich enzyme-linked immunosorbent assay. However, when the tumor is too small, the antigen might not be detected, especially in the blood of patients. Therefore, assay methods that are more sensitive need to be developed. We are trying to apply

thermal reacting magnetic beads and immunochromatography to the detective system.

Analysis of mitochondrial DNA of the genus Nycticebus
Slow lorises (*Nycticebus* ssp.) have been kept, bred, and exhibited for many years in many Japanese facilities. However, because of their endangered status, in 2007 slow lorises were shifted from Appendix II to Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Since then, international trade in slow lorises has been prohibited. However, illegal trade continues. The problem is that when slow lorises are confiscated while being illegally transported, the country of origin and even the name of the species is often unclear. Previously, we determined the species by examining DNA nucleotide sequences, as requested by the Asian Wildlife Research Center. This examination includes amplification, sequencing of mitochondrial DNAs of cytochrome oxidase subunit 1 (COI), and comparison with standard individuals, animals can be classified into the 5 present species of *Nycticebus*. This year, we sequenced the whole mitochondrial DNA of the Bengal slow loris (*Nycticebus bengalensis*). This information will facilitate more accurate identification of the species of each animal.

Functional analysis of 3 novel cell lines derived from human papillary thyroid carcinomas with 3 different clinical courses
Papillary thyroid carcinoma (PTC) is the most frequent thyroid carcinoma. Cell lines derived from PTC have been of considerable value in studying various aspects of thyroid cancer, such as gene expression, cell proliferation, and differentiation. Here we report 3 novel PTC lines established from 3 patients with different backgrounds.
Patient 1 was a 38-year-old woman with a PTC of the right thyroid lobe which had not metastasized. The cell line consisted of epithelial cells with few lysosomes and showed a pavement structure. The secretion of free thyroxine (fT4) and thyroglobulin were increased by thyroid-stimulating hormone or treatment with growth hormone (GH) and insulin-like growth factor (IGF) I.
Patient 2 was a 22-year-old woman with PTC initially in the right thyroid lobe, but 4 years after resection of the right lobe, the PTC had metastasized to the left lobe. This cell line consisted of small epithelial cells with evident lysosomes. The secretion of fT4 and thyroglobulin were slightly increased by TSH or by treatment with GH and IGF-I.
Patient 3 was an 85-year-old man with PTC and acromegaly. The PTC had metastasized to the cervical lymph nodes. This cell line consisted of small epithelial cells with many lysosomes. The cells frequently piled up. The secretion of fT4 and thyroglobulin was significantly increased by treatment with GH and IGF-I. We have established 3 PTC cell lines with substantial variations in phenotype. The cell lines may be useful for thyroid cancer research.

Biophysical characterization of branched amphilic peptide capsules.
Branched amphiphilic peptide capsules (BAPCs) are peptide nanospheres comprised of equimolar proportions of 2 branched peptide sequences — (FLIVI) 2-K-KKKK and (FLIVIGSII) 2-K-KKKK — that self-assemble in water to form bilayer-delimited poly-

cationic capsules capable of trapping solutes. We examined the lipid-like properties of this system including assembly, fusion, solute encapsulation, and resizing by membrane extrusion and also examined their capability to be maintained at a specific size by storage at 4˚C. These studies demonstrated that the capsules, while sharing many properties with lipid vesicles, were much more robust. We also investigated the stability, size limitations of encapsulation, cellular localization, retention, and biodistribution of the BAPCs. We demonstrated that the BAPCs are readily taken up by epithelial cells in culture, escape or evade the endocytotic pathway, and accumulate in the perinuclear region, where they persist without apparent degradation. The BAPCs encapsulated alpha particle-emitting radionuclides without apparent leakage, were taken up by cells, and were retained for extended periods of time. Their potential for clinical application is being examined.

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Department of Allergology

Naohiro Watanabe, *Professor and Director* Hirohisa Saito, *Professor*

General Summary

The Department of Allergology was established in 2011. 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, the dwarf tapeworm, infects humans around the world and also infects mice. Oral infection with eggs of *V. nana* induces strong protection against reinfection with eggs through innate immunity in mice. The protection was observed 1 day after priming infection and had been completed after 2 days, when no challenge worms were detected in the intestine. No protection against oral reinfection was found in mice primed with eggs in the skin or the peritoneal cavity, indicating that priming in the intestine was essential. To examine the specificity of protection, mice infected with 3 species of intestinal nematodes were challenged with *V. nana* eggs. Each nematode infection partially protected against *V. nana* infection, suggesting that the mechanism of protection is likely to be different from that of *V. nana.* Cells responsible for protection were CD4+ T cells, but not natural killer cells, natural killer T cells, basophils, or CD8+ cells. Costimulatory signals were required from inducible T-cell costimulator ligand (ICOSL) but not from CD80 or CD86. These results suggest a novel innate immune system in the intestine for protection against *V. nana*.

Protection by an IgE-level regulatory gene

We have previously reported an IgE-level regulatory gene that causes mice to be IgE high-responders and low-responders. When IgE high-responder mice were infected with *Trichinella spiralis*, and their IgE function was artificially interfered, the protective activity against *T. spiralis* was reduced. However, no reduction in protective activity was found in IgE low-responder mice. These results indicate that an IgE-level regulatory gene controls protection against *T. spirallis* and suggest that a biological role of IgE is protection against parasites.

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Department of Pathophysiology and Therapy in Chronic Kidney Disease

Tatsuo Hosoya, *Professor*
Kimiyoshi Ichida, *Professor*
Iwao Ohno, *Professor*
Keitaro Yokoyama, *Associate 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 rehabilitation of patients who have already undergone HD and to promote home HD (HHD) and continuous ambulatory peritoneal dialysis (CAPD) that can be performed at home. Both HHD and CAPD will greatly benefit patients undergoing HD, particularly patients who have difficulty visiting hospitals because of old age or disability. Furthermore, when the Great East Japan Earthquake occurred, it was shown that CAPD could be performed in disaster areas.

Research Activities

Prevention of CKD and its progression

Hyperuricemia has long be suggested to be a risk factor for the onset and progression of CKD, but definitive evidence was lacking, because an antihyperuricemic agent that could reduce uric acid levels effectively and safely in patients with renal dysfunction, such as CKD, was not available. Within the last 3 years, 2 novel antihyperuricemic agents that can be used effectively and safely in patients with renal dysfunction have been developed. The efficacy and safety of one agent, febuxostat, were investigated in patients with CKD IIIb and IV and reported at academic meetings and in a paper. Furthermore, a double-blind multicenter prospective clinical trial (FEATHER study: Febuxostat versus placebo randomized controlled trial regarding reduced renal function in patients with hyperuricemia complicated by chronic kidney disease stage 3) 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.

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 (The study of evaluating adequateness replacement therapy: EARTH Study) are ongoing as multicenter collaborative studies to elucidate effectiveness of the combination therapy.

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 2 years, and a manuscript is being prepared. It is necessary to verify whether topiroxostat reduces albuminuria similarly in a variety of renal diseases and 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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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 ligase Nedd4 expression and atrophy in unloaded rat plantaris muscle

Two muscle-specific ubiquitin ligases, atrogen-1/muscle atrophy F-box (MAFbx) and muscle RING-finger protein 1 (MuRF1), are prominently involved in skeletal muscle atrophy. Recent studies have suggested that another ubiquitin ligase, neural precursor cell expressed developmentally down-regulated protein 4 (Nedd4), plays a more encompassing role in denervation-induced muscle atrophy. Therefore, we investigated the involvement of Nedd4 in unloading-induced muscle atrophy with a specific focus on age dependency.

We analyzed the fast-twitch plantaris muscle. In a caged control group muscle mass increased from 4 to 10 months and maximum force decreased from 10 to 20 months. Muscle mass and maximum force decreased at any age, but most prominently in 20-month-old rats after 3 weeks of hindlimb unloading. Although the protein expression of MAFbx and MuRF1 was not changed by hindlimb unloading at any age, a significant increase in Nedd4 expression was found only in 20-month-old rats. Intermittent reloading (30 minutes/day, 6 days/week) during the hindlimb-unloading period inhibited muscle atrophy independent of age. However, the effect of reloading on Nedd4 expression was not significant, even in 20-month-old rats. These results suggest that the ubiquitin ligase Nedd4 participates in severe muscle atrophy, at least in old age, although it may not be intimately linked with the atrophy process.

Effects of exercise training and diet restriction

Exercise training and diet restriction are commonly recommended to prevent and ameliorate obesity and lifestyle-related diseases. We previously reported that diet restriction alone exacerbated fatty liver, while diet restriction combined with daily exercise improved fatty liver despite similar food intake in Zucker fatty rats. Fatty acid translocase (FAT) is a key protein regulating the uptake of fatty acids into hepatic cells. Therefore, we examined the effects of exercise and diet restriction on FAT protein expression in the livers of Zucker fatty rats. We found that increases in body weight, serum levels of glucose and free fatty acids, hepatic triglyceride content and lipid droplets, and hepatic FAT expression were suppressed by exercise with diet restriction but were facilitated by diet restriction alone. Therefore, we concluded that hepatic FAT is a possible factor in the

differential effects on fatty liver of diet restriction alone and diet restriction combined with exercise. In addition, we investigated the duration of the effects of exercise training. The above-described effects of exercise disappeared after 2 weeks of detraining and were not long lasting. Therefore, continuation of exercise in daily life might be necessary to maintain one's health.

Exercise-induced improvement in hyperglycemia is mediated by dehydrotestosterone synthesized in skeletal muscle

Exercise decreases hyperglycemia by enhancing glucose metabolism in the skeletal muscle of patients with type 2 diabetes. We have recently reported that sex steroid hormones can be locally synthesized in skeletal muscle to decrease fasting blood glucose levels in obese rats. Here, we determined whether exercise-induced production of sex steroid hormones in skeletal muscle can directly decrease hyperglycemia in Zucker diabetic fatty rats. Thirty rats were randomly assigned to the following groups: control, exercise, or exercise with continuous infusion of 5α-reductase inhibitor via an osmotic minipump. The results indicated that 6 weeks of exercise significantly reduced serum insulin levels and fasting glucose levels compared with those in the control group. Skeletal muscle levels of dehydroepiandrosterone, 5α-dehydrotestosterone, and 5α-reductase were all significantly higher in the exercise group. Moreover, exercise increased glucose transporter-4 translocation with a concomitant upregulation of phosphorylated phosphoinositide 3-kinase and protein kinases B and C-ζ/λ. Furthermore, significant correlations were observed between the fasting glucose level and the muscular dehydrotestosterone level. Correspondingly, the observed exercise-induced improvements in serum insulin and fasting glucose levels were all suppressed by administration of a 5α-reductase inhibitor. These results indicate that the exercise-induced improvements in glucose metabolism signaling and glucose levels may be directly attributable to the increase in sex steroid hormones within skeletal muscles.

Publications

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Department of Cell Physiology
Division of Aerospace Medicine

Susumu Minamisawa, *Professor*
Masamichi Sudoh, *Professor*
Hiroko Toshima, *Associate Professor*

General Summary

Our main research interests are 1) gravitational physiology and aerospace medicine and 2) physioepidemiological studies of health.

Research Activities

Gravitational physiology and aerospace medicine

1. Technique of electrocardiographic recording using medaka

The medaka, or Japanese killifish, is an indigenous model vertebrate of Japan. This fish has various strains, is transparent during embryogenesis, and has been used as a research animal since the 1940s. Experiments with medaka have been performed aboard the International Space Station. Using the transparent medaka strain Sukesuke (SK2), we established a method of detecting the heartbeat and observing heart-rate variability with live imaging under a stereomicroscope. However, because there is no evidence that the live-imaging data is coincident with electrocardiographic (ECG) data, we are developing, in collaboration with the Japan Aerospace Exploration Agency, an ECG technique using medaka.

The medaka was placed in a damp sponge, and bipolar-lead ECGs were recorded under unanesthetized conditions with needle electrodes inserted through the skin. Wave-form analysis was performed with PowerLab data acquisition software (AD Instruments Japan, Tokyo).

In this study, we recorded clear ECG data. Because the data quality might depend on the needle position, a technique for precise needle insertion should be developed.

2. 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.

3. Outreach activities for aerospace medicine

Our outreach activities aim to promote public understanding of science and to provide information to the public and include publishing books and holding public talks, lectures, and discussions. Recently, public outreach has become important in science. We have been starting outreach activities for aerospace medicine.

Physioepidemiological study of health

1. The effect of the musical ensemble on human health

We evaluated the effect of a musical ensemble on autonomic nervous function through use of a respiratory function test and a circulatory function test. Respiratory and circulatory functions were observed to synchronize in the musicians of the ensemble. We believe that this result might lead to the development of a novel musical therapy.

2. Physiological effects of an irregular work schedule on care workers

We measured sleep patterns and the depth of sleep of care workers who have an irregular schedule by using an actigraph. We also measured the circadian rhythm of the autonomic nervous function of these care workers by using Holter ECG and the frequency-analysis method. The sleep pattern and the circadian rhythm were compared.

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 nervous system in 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 analyzed the central nervous system of mouse models of prosaposin deficiency and mucopolysaccharidosis type II (MPS II) with biochemical methods, the amino-cupric-silver method, and immunohistochemical methods with antibodies against accumulating materials, such as SCMAS.
Results: In the central nervous system of mouse models of prosaposin deficiency and MPS II, the numbers of SCMAS-immunoreactive neurons increased in proportion to the amico-cupuric-silver-impregnated neurons.
Discussion: SCMAS is a candidate for amino-cupric-silver-impregnated material in the central nervous system of mouse models of lysosomal disorders.

Detection of point mutations in the isocitrate dehydrogenase gene
Objective: A novel mutation of isocitrate dehydrogenase (IDH) was found in gliomas and in hematopoietic and chondroid neoplasms. Different methods, such as direct polymerase chain reaction (PCR) sequencing, post-PCR fluorescence melting curve analysis, and real-time PCR assays for single nucleotide polymorphisms (SNaPshot, Life Technologies, Carlsbad, CA, USA), and immunohistochemical studies for the IDH1 R132H mutation are available to determine the IDH mutation status. This study assessed the Cycleave PCR method (Takara Bio Inc., Otsu, Shiga, Japan) using chimeric probes containing RNA and DNA for investigating the status of IDH mutations.
Material and methods: We designed chimeric probes and PCR primers for detecting mutations of IDH1 (132R and R132H, R132S, R132C, R132G, R132L, R132V, and R132P) and IDH2 (172R and R172G, R172K, and R172K). For positive and negative controls, we also made recombinant plasmid vectors containing the wild-type IDH gene segments or each mutation. The Cycleave PCRs were evaluated in the various ratios of wild-type

and mutated recombinant plasmid vectors. The assumed tumor condition of 2 IDH mutations was also investigated.
Results: The specificity of Cycleave PCR method for IDH mutations was 100%. The IDH2 R172M probe, containing a palindromic sequence, was unavailable for Cycleave PCR. We could detect more than 0.2% of a single mutation in the wild-type vector. When a tumor contained 2 IDH mutations, not less than 2% of a single mutation was detectable.
Discussion: The Cycleave PCR method can be used for IDH genotyping.

Nuclear functional domain in retinal cells of spinocerebellar ataxia 7
Spinocerebellar ataxia 7 (SCA7) is an autosomal dominant neurodegenerative disorder characterized by cerebellar ataxia and retinal degeneration. SCA7 is caused by a polyglutamine expansion in ataxin 7. The pathologic hallmark of SCA7 is the formation of neuronal intranuclear inclusions (NIIs) through the accumulation of mutated ataxin 7. Nuclear functional domains related to the formation of NIIs, especially promyelocytic leukemia nuclear bodies, could be accumulation sites of the pathological ataxin 7 with expanded polyglutamine. Spliceosomes and Cajal bodies could be related to the formation of NIIs in SCA7. In this study, we examined the relation of spliceosomes and NIIs in the retina of SCA7 knock-in mice. Ataxin 7-immunoreactive NIIs were present in all cell layers of degenerated SCA7 knock-in mice retina. In the retina of wild-type mice, NIIs immunoreactive for coilin (a molecular marker of Cajal bodies) and sm (a marker of spliceosome) were observed in the ganglion and internal granular cell layers. In SCA7 knock-in mice, sm-immunoreactive structures were found in the external granular cell layer of the retina, and coilin-immunoreactive structures were unchanged. These findings indicate that alteration of the nuclear spliceosome functional domain is related to RNA metabolism in retinal cells in SCA7.

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Reviews and Books

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Department of Orthopaedic Surgery
Division of Sports Medicine

Keishi Marumo, *Professor* Hiroki Funasaki, *Associate Professor*

General Summary

Clinical Research
In our ongoing research we focus on competitive athletes (including professionals), amateurs who include sports activities in their daily lives, and young athletes engaged in school sports clubs or dedicated to training within sports clubs. We have incorporated basic research since 2013.

Research Activities

Bone quality in female ballet dancers
We investigated bone quality-related markers by measuring serum levels of homocysteine and pentosidine, bone metabolic markers, and bone mineral density (BMD) in 13 elite female ballet dancers (average age, 22 years) to evaluate a possible correlation between amenorrhea or fatigue fracture or both. Two dancers had a history of a fatigue fracture, and 3 had a history of secondary amenorrhea. Although none had abnormal BMD, serum levels of bone alkaline phosphatase, tartrate-resistant acid phosphatase 5b, and homocysteine, in 2 of the 3 dancers with a history of secondary amenorrhea had abnormally high levels of serum pentosidine. We speculated that oxidative stress related to an increased mechanical load might lead to impaired estrogen secretion, which eventually affects bone quality. We speculated that latent bone quality deterioration might develop in female athletes with secondary amenorrhea but otherwise normal BMD values.

The silent period in patients who had conservative therapy for anterior cruciate ligament injury: Comparison between the affected and unaffected knees
The purpose of this study was to compare premotion time, the premotion silent period, and the switching silent period, motor unit functional parameters, between affected and unaffected knees in patients who had received conservative treatment for anterior cruciate ligament (ACL) tear. Seven patients were enrolled. They were examined at an average of 6 months after the injury. There were no significant differences in the premotion time, the premotion silent period, and the switching silent period between the affected and the unaffected sides. A previous study demonstrated that the premotion silent period and the switching silent period on the operated side were longer than those on the unoperated side in patients after reconstruction surgery for the ACL tear. Our results suggest that the nerve-muscle coordination in conservatively treated patients might recover more quickly than in patients treated with surgery.

Muscle strength after reconstructive surgery for ACL tear
Muscle strength of the quadriceps and hamstring muscles in 60 patients was measured 4 and 8 months after reconstructive surgery for an ACL tear. Muscle strength on the operated side increased significantly between 4 and 8 months after surgery with a constant quadriceps:hamstrings ratio. However, patients with marked muscle weakness at 4 months did not acquire sufficient muscle strength by 8 months. More exercises during rehabilitation or a delay in sports activities or both are necessary in these patients.

Rotational range of motion of the shoulder joint in patients with throwing pain
We used ultrasonography to measure the 2nd rotational range of motion of the shoulder after exclusion of the humeral head retroversion angle (the true rotational range of motion) in 27 patients with throwing pain. These patients showed significantly increased true external rotational range and significantly decreased true internal rotational range. The total rotational range of motion was significantly decreased. Retroversion of the humeral head, tightness of the posterior soft tissues, and eccentric contraction of the anterior soft tissues might influence the true rotational range of motion of the shoulder joint.

Arthroscopic excision of bone fragments in a neglected fracture of the lateral process of the talus in a junior soccer player
We reported on an 11-year-old male soccer player who had sustained a fracture of the lateral process of the talus 6 months earlier and then underwent arthroscopic excision of fragments of the talar lateral process. The osseous overgrowth was resected piece by piece, and the loose body was removed en bloc. The patient resumed playing soccer 5 weeks after the operation. This case exemplifies 2 important points: 1) this type of fracture can develop even in children and not only in snowboarders; 2) the arthroscopic excision of fragments of the talar lateral process can be accomplished easily and facilitates an early return to activity.

Bone quality in neurofibromatosis type I
We examined BMD and markers related to bone quality in 17 patients with neurofibromatosis type I. Three patients had osteoporosis, and 4 patients had high serum levels of pentosidine. Although these increased pentosidine levels were not correlated with bone manifestations, further longitudinal investigations of bone metabolism in patients with neurofibromatosis type I are needed.

Publications

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Reviews and Books

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Health-Care Center

Mikio Zeniya, *Professor and Director*
Takashi Wada, *Professor*
Hiroki Takahashi, *Assistant Professor*
Yuhshi Kuniyasu, *Assistant Professor*
Masanobu Kaji, *Professor*
Takekazu Onda, *Professor*
Masako Iwanaga, *Assistant Professor*
Hiroko Nogi, *Assistant Professor*

General Summary

The most outstanding event was Dr. Wada's winning a prize for an excellent article in 2013 from the Japanese Society of Health Evaluation and Promotion. The article was entitled "Of the three classifications of healthy lifestyle habits, which one is the most closely associated with the prevention of high blood pressure?" (HEP 2013; 40: 457-463). We have investigated the proper guidance and extraction of problems in specific health guidance with the support of a Health and Labour Sciences Research Grant.

Research Activities

Healthy lifestyle habits and prevention of high blood pressure

A healthy lifestyle often prevents high blood pressure. To date 3 kinds of simple healthy lifestyle mottoes for everyone, reported proposed by Breslow' 7 items, Morimoto's 8 items, and Ikeda's 6 items, have been reported. However, to our knowledge, no study had investigated the association between the incidence of high blood pressure and the practice of these health habits. The objective of the present study was to determine which of the 3 classifications of Breslow, Morimoto and Ikeda is most closely associated with the prevention of high blood pressure. The cumulative 9-year incidence of high blood pressure was calculated and compared among these classifications using the log-rank test adjusted for age in the present open retrospective cohort study. A total of 5,884 subjects undergoing medical checkups and answering a self-administered questionnaire about the above healthy lifestyle habits were divided into poor, moderate, and favorable lifestyle groups. High blood pressure was defined as a systolic blood pressure ≥130 mm Hg, a diastolic blood pressure ≥ 85 mm Hg, or treatment with antihypertensive agents during follow-up.

For Breslow's habits, the incidence of high blood pressure was higher in the favorable group than in the poor and moderate lifestyle groups for males. For Morimoto's habits, there were no significant differences in the incidence among the 3 lifestyle groups. For Ikeda's habits, the incidence of high blood pressure in the poor lifestyle group was significantly higher than in the favorable and moderate lifestyle groups for males, although the habits were not predictive for females. Our results suggest that Ikeda's healthy habits decrease the risk of high blood pressure.

Significance of visual field tests in medical checkups

The purpose of the present study was to investigate the potential benefits of visual field tests over standard noncontact measurements of intraocular pressure commonly used in

medical checkups to detect glaucoma. From April 2010 through March 2011, we screened 6,453 individuals who underwent annual medical checkups. Thirty-four subjects (0.5%) were found to have an intraocular pressure of 22 mm Hg or higher. Seven of these subjects (0.1%) had previously been found to have glaucoma, while another 2 subjects (0.03%) were newly found to have glaucoma. Of the 683 subjects (10.6%) found to have visual field abnormalities, 309 (4.8%) had previously been found to have glaucoma. The remaining 374 subjects (5.8%) without glaucoma were followed up until March 2012. During follow-up, 66 subjects (1.0% of the total medical checkup subjects) were newly found to have glaucoma, and 63 subjects (1.0%) were found to have other eye diseases. Forty-eight subjects (27% of the subjects who visited an ophthalmologist) exhibited no abnormalities during follow-up, but we had no information about 197 subjects who did not visit an ophthalmologist or undergo any further comprehensive examination. This study found a significantly higher percentage of newly diagnosed glaucoma cases with the visual field test (1.0%) than with noncontact measurement of intraocular pressure (0.03%). Our results demonstrate the importance of adding a visual field test to the standard eye examinations used in medical checkups.

Publications

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Premedical Course

Biology

Koji Takada, *Professor* Rie Hiratsuka, *Associate Professor*

General Summary

Our research themes are to understand the mechanisms of heavy metal toxicity and the abnormal behaviors of mice in terms of the ubiquitin-conjugating system, and the reproductive system of plants, particularly pollen development in Japanese cedar.

Research Activities

Involvement of protein aggregates in cell toxicity of heavy metals
Intracellular protein aggregates are accumulated by exposure to the toxic heavy metals. Effects of cadmium (Cd) on proteins in kidney HK-2 cells were analyzed. After the cells were cultured with $CdCl_2$ for up to 12, 24 and more than 48 hours, the median effective concentrations for cytotoxicity were estimated to be 200, 85, and 70 μM, respectively. Amounts of the aggregates, quantified with the enzyme-linked immunosorbent assay for polyubiquitin, were low during exposure to up to 40 μM Cd, which produced no toxic effects. In contrast, by the exposure to more than 70 μM Cd, the aggregates were augmented in the cells, and prolonged accumulation was observed only in the sublethal condition.

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 which allows for retraction from unavoidable stresses. To identify the targets of USP46 in neuronal cells, two cell lines expressing wild-type or mutant USP46 fused with FLAG peptide were constructed, and several proteins, including WD repeat domain 48 and dystrophia myotonica WD repeat-containing protein, were obtained from the cells via immunoprecipitation with the FLAG antibody-agarose and liquid chromatography/tandem mass spectrometry analysis.

Histological study of pollen development in male sterile Cryptomeria japonica
Cryptomeria japonica pollinosis, caused by the Cry j 1 and 2 proteins, affects more than 20% of the Japanese population. To investigate the mechanisms causing male sterility of *C. japonica* (Shindai 8), histological information was collected. At the end of October, Cry j 1 and 2 were detected in the microspores. In mid-November, each microspore divided normally to form a small generative cell and a large tube cell. However, following the division, the pollens underwent cell death, with vacuole expansion, organelle deg-

radation, and defective cell walls. The allergens disappeared in the dying cells.

Publications

Sugimoto S, Iwamoto T, Takada K, Okuda K, Tajima A, Iwase T, Mizunoe Y. Staphylococcus epidermidis Esp degrades specific proteins associated with Staphylococcus aureus biofilm formation and host-pathogen interaction. *J Bacteriol.* 2013; **195:** 1645-55.

Iwase T, Tajima A, Sugimoto S, Okuda K, Hironaka I, Kamata Y, Takada K, Mizunoe Y. A simple assay for measuring catalase activity: a visual approach. *Sci Rep.* 2013; **3:** 3081.

Physics

Tsuyoshi Ueta, *Professor*

Katsumi Kasono, *Assistant Professor*

General Summary

1. We have proposed a metal photonic crystal with lattice vibration as a system enhancing the dynamic Casimir effect, and have been investigating the properties of the dynamic Casimir effect within a metal photonic crystal.
2. Computer simulation of phase transitions, critical phenomena, and interacting many-body systems.

Research Activities

Numerical study of the structural color of blue birds

The color of some birds, such as the kingfisher and the red-flanked 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-flanked bluetail; the optical property of the model was numerically analyzed and compared with that of the structural color.

Monte Carlo simulation of the ferromagnetic Potts models

We calculated the discontinuity of magnetization m at the transition temperature of the first-order phase transition. Cluster Monte Carlo simulations were used to study 10 state ferromagnetic Potts models on the kagome, 4-8, generalized square lattices. It appears that m has no universality between the different lattices.

Publications

Fujii G[1], Watanabe H[2], Yamada T[2], Ueta T, Mizuno M[1] ([1]Akita Pref Univ, [2]Koto Univ.). Level set based topology optimization for optical cloaks. *Appl Phys Lett.* 2013; **102:** 251106.

Ueta T. Enhancement of the dynamic Casimir effect within a metal photonic crystal. *Proceedings of SPIE.* 2013; **8771**: 17-8.

Fujii G[1], Ueta T, Mizuno M[1] ([1]Shinshu Univ). Finite element analysis for laser action in porous random media. In: Proceedings of Metamaterials

2013: 7th International Congress on Advanced Electromagnetic Materials in Microwaves and Optics; 2013 Sep 16–19; Bordouex, France. Piscatway: IEEE; 2013. p. 334–6.
***Fujii G*[1], *Watanabe H*[2], *Yamada T*[2], *Ueta T, Mizuno M*[1] (*[1]Akita Pref Univ, [2]Nagoya Univ).** Level set based topology optimization for optical cloaks containing a large scattering object. In: Proceedings of 10th WCSMO 2013: 10th World Congress of Structural and Multidisciplinary Optimization; 2013 May 20–24; Orlando, USA. Lisboa: ISSMO; 2013. p. 5283.

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 ^{13}C-labeled materials for metabolic and diagnositic research

^{13}C-Labeled biologically active compounds are useful as probes for metabolic and diagnotic research because they can be directly applied to mass spectrometry or infrared spectroscopy without separation or purification. We are engaged in the synthesis of ^{13}C-labeled galactosyl benzyl glycoside and $^{13}C_{20}$-retinol. Galactose pentaacetate was reacted with α-^{13}C-benzyl alcohol in the presence of boron trifluoride etherate to produce its α-glycoside. Although the synthesis of $^{13}C_{20}$-retinol has already been reported, the reproducibility was not satisfactory, and a new synthetic route was explored.

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 or dimethylsulfoxide.

Publications

***Hashimoto C, Sugimoto K*[1], *Takahashi Y*[1], *Kodomari M*[1] (*[1]Shibaura Inst Techol).** An efficient method for the synthesis of phenacyl ester-protected dipeptides using neutral alumina-supported sodium carbonate 'Na2CO3/n-Al2O3'. *J Pept Sci.* 2013; **19:** 659–62.

Reviews and Books

Okano T. Heterocyclic synthesis via catalysis of

N-heterocyclic carbenes: very classical and very modern chemical species. *Heterocyclic Communications.* 2013; **19:** 311–26.

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 2013.

Reviews and Books

Ozawa R. Kenpou wo Manabi Ikashi Mamoru. Tokyo: Gakushu no Tomosha; 2013.
Ozawa R. The disposition of HLW and law & democracy (in Japanese). In: Hirowatari S, Asakura M, Imamura Y, editors. Nihon shakai to shimin hogaku. Tokyo: Nihon Hyoronsha; 2013. p. 211–28.
Ozawa R, Tanaka T, Yamaguchi N. Shimin ni senkyo wo torimodose! (in Japanese). Tokyo: Ohtsuki Shoten; 2013.

Human Science

Takao Fukuyama, *Professor*

General Summary

The study of Western philosophy and ethics

Research Activities

Essential Encounter
An encounter provides an effect, which derives from meeting others whom we had longed for. Such others awaken us to the subject and possibility of our lives and help us to realize them. But how do the others help us? They help us because they show us concrete values, which are not described in the abstract but are in vivid action. From this encounter arises a new communion. We give something to a person, who then gives us something else in return.

Value of attitude
The ethics of responsibility also provides a kind of answer about one's views of life and

death. Viktor Frankl, the founder of logotherapy, proposed the concept of attitudinal value. When a person is bedridden, he cannot act freely, but he can consider the feelings of others. Frankl thought that the attitudinal value is the most important of all values. Frankl suffered a cruel fate at the Auschwitz concentration camp. He had nothing free there, but he could maintain a proud-hearted attitude.

Japanese

Ikuko Noro, *Professor*

General Summary

1. To study patients' perception of shared decision-making
2. To study the validity of applying the Roter Interaction Analysis System to analyze communication between nurses and patients with mental disease

Research Activities

To study patients' perception of shared-decision making
Based on the research on the perception of patients with cancer on shared decision-making, we reported that: 1) patients who perceived the decision-making process as shared collected and received more information from their physicians than did patients who perceived their decision-making as physician-centered, and 2) the differences among decision-making processes did not affect patients' satisfaction; however, patients whose preferred decision-making and perceived decision-making processes matched were more satisfied than were patients whose processes did not match.

The validity of applying the Roter Interaction Analysis System to analyze communication between nurses and patients with mental disease
We used the Roter Interaction Analysis System to analyze conversations between nurses and patients with depression or with schizophrenia. We found that 1) the utterances of patients with depression or schizophrenia were almost as many as those of nurses, and 2) nurses asked few questions of patients, but the nurses frequently showed agreement.

Publications

Noro I, Kawano M. Analyzing depression patient-nurse communication and schizophrenia patient-nurse communication by using Roter Interaction Analysis System (RIAS) (in Japanese). *Seishin Kango ni okeru Discourse Bunseki Kenkyu Kaishi.* 2014; **2:** 15-21.

Reviews and Books

Noro I. Documents for informed consent (in Japanese). In: Ishizaki M, Noro I, editors. The prospect of medical communication. Tokyo: Shinohara Shuppanshinsha; 2013. p. 117-24.

Mathematics

Katsuya Yokoi, *Professor*

Hiroshi Shiraishi, *Assistant Professor*

General Summary

1. To study dimension theory and topological dynamics
2. To consider the asymptotic behavior of estimators of optimal portfolios when the return processes are various stochastic processes.

Research Activities

1. We studied omega-limit sets and (strong) chain recurrent sets on topological dynamics.
2. We examined the estimation of optimal portfolios when the return processes are continuous-time stochastic processes. We also examined the estimation of optimal portfolios with a large number of assets.

Publications

Yokoi K. Recurrence properties of a class of nonautonomous discrete systems. *Bulletin of the Belgian Mathematical Society-Simon Stevin*. 2013; **20:** 689-705.

English

Osamu Ohara, *Professor*

Tetsuro Fujii, *Associate Professor*

General Summary

English audiovisual 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 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.
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 studied commonality between English and Japanese and identified the types of English vocabulary that is conducive to learning.

Research Activities

Ohara presented a paper at a session in the International Medieval Congress 2013 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 second textbook in the series, *World Trek — English Communication II*, and its instructional aids, *World Trek — English Communication II Teacher's Book* and *World Trek — English Communication I Teacher's Manual*, were published.
Fujii presented about common words and collocations between English and Japanese in "Investigating similarities between English and Japanese" at the 9th yearly conference held by the Association for Japanese and English Language and Culture in Tokyo in June.

Reviews and Books

Mochizuki M[1], Aizawa K[2], Allum P[3], Sasabe N[4], Hayashi Y[5], Fujii T, Miura S[6] ([1]Reitaku Univ, [2]Tokyo Denki Univ, [3]Rikkyo Univ, [4]Toritsu Aoyama High, [5]Soka High, [6]Tsurubunka Univ).
World Treck English Communication II. Tokyo: Kirihara Shoten; 2014.

Mochizuki M[1], Aizawa K[2], Allum P[3], Sasabe N[4], Hayashi Y[5], Fujii T, Miura S[6] ([1]Reitaku Univ, [2]Tokyo Denki Univ, [3]Rikkyo Univ, [4]Toritsu Aoyama High, [5]Soka High, [6]Tsurubunka Univ).
World Treck English Communication II: Teacher's book. Tokyo: Kirihara Shoten; 2014.

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.

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 4th 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.

Publications

Baba K, Saito M, Tanaka S, Maruyama Y. Relationship between job satisfaction and work environment of certified nurse specialists working in hospital (in Japanese). *Nihon Kango Kenkyu Gakkai Zasshi.* 2013; **36**(2): 95-104.

Nursing Administration

Midori Nagano, *Professor*

General Summary

Three studies have been performed: **"**Nursing manager's support for a wound ostomy continence nurse in pressure ulcer measures at a hospital," "Requirements at a stoma clinic in a hospital," and "Health work environment in nursing practice."

Research Activities

Manager's support for a wound ostomy continence nurse in pressure ulcer measures at hospital

To clarify the subjective effects of wound, ostomy, and continence nurses (WOCN) on pressure ulcer management systems at their institutions, on the structural requirements, and on effective measures in pressure ulcer management related to the incidence of pressure ulcers, a questionnaire survey was conducted at 425 hospitals that gave consent. The subject of analysis was 166 replying hospitals having 200 beds or more. Our findings indicate that by using the perception of influence as a measure of achievement, the quality of pressure ulcer management, which is not reflected in the incidence of pressure ulcers, can be assessed.

Requirements at a stoma clinic in a hospital

The present study aimed to clarify trends, issues, and current requirements at a stoma clinic in a large hospital to contribute to the development of a sustainable system to better support the needs of persons with ostomies. We found that the median age of patients with stomae was continuing to rise. We attribute this greater median age to 1) more low anterior resection procedures, 2) more procedures to create temporary ileostomies, and 3) shorter hospital stays.

Health work environment in nursing practice

We examined previous researches of health work environment in nursing practice. Joint research with concerned parties for the explication of the health work environment in nursing practice in Japanese hospitals was performed with a questionnaire survey.

Publications

Nagano M. Manager's support for a wound ostomy continence nurse in pressure ulcer measures at hospital (in Japanese). *Nihon Sosho Ostomy Shikkin Kanri Gakkaishi.* 2014; **17:** 281–5.

Adult Nursing

Shoko Fujino, *Professor*
Hiroaki Murata, *Assistant Professor*
Ako Terakado, *Assistant Professor*
Naomi Takashima, *Professor*
Ruka Seyama, *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 performed with an emphasis on the process of learning practical nursing skills through chronic-phase and perioperative nursing practicums. As part of their research activities, faculty members explored cancer nursing topics and nursing care for acutely and critically ill patients. In the field of adult nursing, practical training with an emphasis on nursing process development is evolving to help students acquire problem-solving skills based on an understanding of the target subject. Based on the previous school year's practicum evaluations, we aimed to improve both classroom and practicum education. As a result, there were positive effects on student involvement and nursing process development. In addition, a shared learning effect was achieved by holding a summarizing conference on the final day. While student evaluations of the 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. For this reason, we introduced paper and video patients in the classroom and offered hands-on education that included the management of stoma or drip intravenous, resuscitation, and electrocardiogram diagnosis, which are essential nursing skills for assessment and intervention. Moreover, practicum education was offered after individual feedback was provided on each course. In the practicum environment and organizational arrangements, cooperation with clinical practicum instructors was strengthened by setting up opportunities for students to review their 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 critiques in a timely manner, providing insights into nursing-process development based on records, conducting nursing practice with students, and ensuring safety. These are aspects that we hope to continue, and practicum training with appropriate interactions is also anticipated in the future by adjusting the division of roles of the parties involved.

Research Activities

Research on Cancer Nursing

1. Development of a nursing support program for patients with dementia and cancer

Behavioral and psychological symptoms of dementia sometimes cause significant problems in nursing for patients with dementia and cancer. Examples include the communi-

cation and assessment of symptoms and the evaluation of nursing intervention. Therefore, we decided to develop a nursing support program for patients with dementia and cancer. This year, 24 nurses were interviewed to find out more about the care of patients with dementia and cancer. We found that the nurses had been an effort to better assess the individual of the patients. However, they had to help patients while facing [anxiety that does not always disappear]. In the future, we plan to clear valid assessment tools and support of patients with dementia and cancer and proceed with the evaluation research.

2. Research on support for patients with cancer undergoing outpatient radiation therapy and their families

We are developing a support system to deal with the psychosocial distress of patients with cancer undergoing outpatient radiation therapy and their families. Last year, on the basis of a literature review we developed the hypothesis that certain effects can be obtained by performing group therapy after the completion of radiation therapy. Therefore, we have studied properly and feasibility in practicing the group therapy with clinician. As a result, we found that the support for patients with cancer and their families was necessary to be implemented educational intervention by specialists not only group therapy. As a result, if educational intervention with thematic, it becomes verifiable. In the future, we will discuss the contents of the program to develop a model intervention.

3. Research on chemotherapy-induced peripheral neuropathy

In collaboration with other institutions, we have been developing an assessment tool for chemotherapy-induced peripheral neuropathy. This year, we worked with clinicians to verify the reliability and validity of the assessment tool. In addition, we submitted a research plan, which was reviewed by the ethics committee. We are performing a survey.

Research on critical care

1. The development of the comfort 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 95 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 urgent hospitalization and intubation time, a lack in the past disease, and the degree of sedations. We would like to propose a nursing support program for the comfort care of patients in the ICU.

2. The postoperative quality of life of patients with gastric cancer

We examined the relationships between physical activity performed during the perioperative period by patients undergoing gastrectomy for gastric cancer and such factors as life condition, psychological condition, and health-related quality of life (QOL). Factors related to physical activity were preoperative activities, exercise habits, pain, dietary intake, coexisting disease, blood albumin levels, motivation in performing activities, con-

fidence in performing activities, and the vitality and physical component summary scores of the QOL scale. At postoperative outpatient visits, it is important to examine symptoms and to perform team-based assessment and treatment of the patients' physical and psychological conditions, including dietary intake, physical activity, and fatigue.

We treated a patient in whom delayed gastric emptying and dilatation of the remnant stomach developed despite the absence of structural or morphological abnormalities in the early phase after Roux-en-Y reconstruction following distal gastrectomy. Multiple factors, including the underlying disease, dietary habits, and psychological factors, were thought to have been involved in the onset of the present case.

It was considered necessary to regard outpatient care during the early postoperative phase as a period of "recovery of stamina" and "dietary rehabilitation" and to conduct careful and continuous follow-up.

3. The development of a nursing support program for patients receiving ventilatory support

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 (NPPV). Characteristic among these were "treatment experiences that changed as time elapsed" and "coping (with it) on my own by trial and error." Notably, these experiences differed from those of patients for whom endotracheal tubes had been inserted, in that patients undergoing NPPV maintained their will to continue NPPV with the mindset of "this can fit into my lifestyle." We continue data collection and will evaluate the effect of intervention on the development program.

4. Clarifying the clinical "knowledge" Clinical intellect of a nurse buried in nursing practice called the physical restraint of the nurse

We are searching the structure of the development of the clinical "knowledge" of nursing students, novices, and expert nurses to visualize nurses' clinical "knowledge" Clinical intellect of a nurse buried in nursing practice called the physical restraint of the nurse.

First, we qualitatively analyzed the process of learning in nursing related to the physical restraint of the nursing student. As a result, the student learned that I settled by oneself while considering the feelings of a patient and the family for rich sensitivity, and conflicting in yourself by susceptible ethical sensibility. In addition, the belief that temporary estraint was necessary was formed from the viewpoint of safety management in critical care while becoming a nurse, and being conscious of the role as the organization man, the responsibility, and it became clear that the nursing care that student wanted to provide began to be develop.

We will examine the development of clinical "knowledge" that formed during nurse's training and related factors.

Publications

Takashima T, Nakada K, Watanabe C, Murata H, Kawai M, Ozone M, Mitsumori N, Kobayashi K, Omura N, Kashiwagi H. Physical actibity insreases significantly from after discharge to 2 months after surgery in patients undergoing gastrectomy for gastric cancer (in Japanese). *Tokyo Jikeikai Ika Daigaku Zasshi.* 2014; **129:** 1-9.

Watabe S, Takeda N, Takashima N. Evidence of pre-operative skin care for total hip arthroplasty and its association with infection control systems in Japan (in Japanese). *Nihon Undoki Kango Gakkaishi.* 2013; **8:** 48-56.
Kawai M, Nakada K, Kawamura M, Yano F, Shida A, Mitsumori N, Omura N, Takashima N, Yanaga K. A case of dilatation of the remnant stomach due to food adjustment disorder early after gastrectomy (in Japanese). *Tokyo Jikeikai Ika Daigaku Zasshi.* 2013; **128:** 229-34.

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 with dysphagia and pressure ulcers. Methods of training nursing students have changed in the revised 2012 curriculum; therefore, we are developing training hospitals and institutions and continuing discussions on specific training methods in the field of geriatric nursing.

Research Activities

Research activities in our field that we have been engaged in are as follows.
1. A study in which elderly residents of nursing homes were instructed on methods of exercising: the results were collected and published with the aim of improving lower-limb edema in elderly residents who use wheelchairs.
2. A study among students who have completed training of learning through lectures, seminars, and training and of the achievement of nursing skills to prevent pressure ulcers. The title of the study was "An investigation of basic nursing education content in 'techniques for preventing pressure ulcers and promoting their healing.'"

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. We presented an interim report based on national data at a Congress of the International Federation of Psychiatric Epidemiology and have finished analyzing new data in 2013. Moreover, we are also researching the human caring approach and have continued to perform discourse analysis. Furthermore, the Okuma Award for insomnia research in 2013 was given to our paper "Insomnia as a mental effect on the general population after the Great East Japan Earthquake", this research was supported by a Grant-in-Aid for Scientific Research.

Publications

Katsuki T, Shioda K, Mitsui M. Insomnia as mental effects on general population after the Great East Japan Earthquake (in Japanese). *Fumin Kenkyu*. 2013; **2013**: 15-22.

Child Nursing

Kiyo Hamanaka, *Professor*

Kinu Takahashi, *Associate Professor*

General Summary

Sharing the outcomes of our study, "Participating and learning of students in a nursing program on children's health promotion at an outpatient department," with other researchers by publishing it in an academic journal has been meaningful. With the results of analysis being systematically reported, another study, "Demands and expectations of members of the Association of Parents of Children with Incurable Diseases regarding Research into Treatment for Specific Chronic Child Diseases," has contributed to the activities of parents' associations and improvements in social systems. A third study, "Participation of pediatric nurses in ethical education programs and nursing approaches to protect children's rights: findings from their free descriptions regarding notable contents and events," has also been significant in providing future perspectives on nursing ethics and approaches to protect children's rights as part of basic nursing education and postgraduate training. Similarly, our fourth study, "Process of establishing in pediatric nurses internal factors associated with the protection of children's rights," has enhanced awareness of the protection of children's rights by pediatric nurses by examining the applicability to pediatric nursing practice and education the theory of this study.

Research Activities

Participating and learning of students in a nursing program on children's health promotion at an outpatient department

The results of a study, conducted 2 years earlier to clarify the learning experiences of stu-

dents who had participated in a nursing program to promote pediatric outpatients' health, were reported in an academic society journal. In this study, the contents of reports submitted by 20 students of a nursing university were qualitatively analyzed, and 8 categories, including "insight into target children" and "communication when practicing the program," were extracted and confirmed the positive learning effect of the study program on students and the possibility of the further development of outpatient nursing.

Demands and expectations of members of the Association of Parents of Children with Incurable Diseases regarding Research into Treatment for Specific Chronic Child Diseases

As we did last year, this year we conducted a survey of members of the Association of Parents of Children with Intractable Diseases to clarify their use of, expectations for, and demands for programs, such as Research into Treatment for Specific Chronic Child Diseases, or support systems, and qualitatively analyzed the results. Analysis revealed diverse contents, including the parents' true emotions, a series of problems associated with demands and expectations, and individuality, and highlighted the necessity of urgent approaches and support.

Participation of pediatric nurses in ethical education programs and nursing approaches to protect children's rights: findings from their free descriptions regarding notable contents and events

In the present study, the notable contents and events freely described by nurses were qualitatively analyzed. In basic nursing education, they learned about a wide range of issues; however, the description of their contents was frequently limited to simply listing the names of topics or items. In postgraduate training, the nurses frequently learned about ethical issues specific to children.

Process of establishing in pediatric nurses internal factors associated with the protection of children's rights

A qualitative and inductive study adopting the grounded theory method was performed to clarify the process of establishing in pediatric nurses internal factors associated with the protection of children's rights, and "developing an insight into target children" was extracted as such a process.

Publications

Ishii M, Hamanaka K, Oikawa I, Kawaguchi C, Hasegawa K, Yamamoto M, Asano H, Yanase J. Participating and learning of the students in the nursing program on "Children's Health Promotion" at an outpatient department (in Japanese). *Nihon Shoni Kango Gakkaishi*. 2103; **22**(2): 9–16.

Omi S, Tsubomi R, Okada S, Hongo T, Kawai Y, Kinjo Y, Miyagishima K, Suzuki E, Hamanaka K. A national survey of physicians' efforts to provide educational support to children with cancer: focusing on cooperation between family, nurses and school teachers (in Japanese). *Nihon Shoni Ketsueki Gan Gakkai Zasshi.* 2013; **50:** 598–606.

Maternity Nursing

Kimiko Kayashima, *Professor* Yasuko Hososaka, *Associate Professor*

General Summary

Studies have been performed to examine the various health issues in each of the lifestyle stages of women and to explore how nursing assistance should be extended in maternal nursing.

Research Activities

Female sexual function and influencing factors in women 4 to 5 months postpartum
To investigate the recovery of sexual function during the postpartum period and factors influencing sexual function, women visiting a clinic for postnatal health checks (4-month postnatal health check) from June through August 2010 were asked to fill out a questionnaire. The average age of respondents was 32.3 ± 4.6 years, whereas the average time since childbirth was 18.6 ± 1.9 weeks. Of the respondents, 52 (44.8%) had just given birth to their first child and 64 (55.2%) had already given birth before their latest pregnancy. Eighty-two respondents (53.9%) reported resuming sexual relations at a mean time of 10.0 ± 4.7 weeks after birth. The average total score on the Female Sexual Function Index was 21.77 ± 5.83, and the scores for the domains of sexual desire and sexual arousal were lower than those for other domains. The total Female Sexual Function Index score was significantly higher and sexual function was better in women who had started menstruating, received help with child rearing, felt well physically, had no concerns over restarting sexual activity, and had low childcare anxiety.

Survey of current newborn cleaning care using a mixed-methods approach
The purpose of this study was to use a mixed-methods research to clarify factor influencing the selection of early infant cleaning care and to explore the ideal method of infant cleaning care. An explanatory mixed-methods design was used. Quantitative data were sent to obstetrics facilities across Japan for self-administered questionnaires to be sampled with probability proportionate to size. In the qualitative survey, semistructured interviews were conducted for 5 obstetric nurses practicing infant cleaning care, and verbatim records were used to perform qualitative and inductive analyses. Responses were collected from 256 institutions in the nationwide cross-sectional survey. Most respondents opted for a dry technique (65.2%) for cleaning on the day of delivery and for bathing (74.9%) from first day after delivery. The number of deliveries or the midwife's efforts did not affect the selection of cleaning care. However, there were significant differences according to the number of staff ($p < 0.01$).
As for cleaning care in facilities in which the subjects of the qualitative survey were engaged, 2 subjects selected bathing and 3 selected a dry technique. Four categories were extracted, namely, "infant-centered cleaning care," "parent-centered cleaning care,"

"cleaning care in consideration of medical personnel's burden," and "switching between the 2 infant-cleaning techniques." The bathing or the dry techniques are defined by the facility cleaning care of current. In this study, the selection of the ideal method of infant cleaning care depended on the state of the infants and the hope of mother.

Functions and roles of maternal transport coordinators in Japan
We conducted a survey of maternal transport coordinators in Japan's 47 prefectures and found that 14 prefectures have instituted a coordinator system. Most of the facilities that had coordinators were medical institutions. Most coordinators were physicians or midwives. Of these coordinators, some only arranged transportation to hospitals, whereas others also called the medical emergency service and arranged transportation and transfer to hospitals. According to the investigation results by region, coordinator systems tended to be more common in larger cities.

Publications

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Community Health Nursing

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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.

Evaluation of Community Health Nursing Practicum
This study aimed to evaluate how the improved Community Health Nursing Practicum II

has influenced learning by nursing students at the university. The law regarding nursing, public health nursing, and maternity nursing schools in Japan was revised in 2008 and 2011. As a result the community nursing course in the education programs for nurses and public health nurses in this department were also changed to 6 subjects, 10 units, and 180 hours and 3 practicums, 4 units, and 180 hours, respectively (i.e., Community Health Nursing Practicum I, II, and III). Community Health Nursing Practicum II, which was introduced in 2011, provides opportunities to learn about the roles of public/occupational/school health nursing through visits to political offices, companies, health insurance associations, elementary schools, and junior high schools.

Exploring career anchors 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.

Reflection among newly appointed public health nurses in community nursing

The purpose of this study was to clarify the characteristics of contents promoting reflection among newly appointed public health nurses in community nursing and to thereby obtain suggestions for methods of promoting reflection that enhances the expertise of newly appointed public health nurses. In this study, we found that reflection by public health nurses was promoted by their first year working for local governments.

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, Takahashi I, Sasai Y. Characterizing student's learning attitude when practicing community health nursing (in Japanese). *Tokyo Jikeikai Ika Daigaku Zasshi.* 2013; **128:** 109-19.

Takahashi I, Shimasawa J, Kubo Y, Sasai Y. Student's recognition for educating public health nursing in a university of nursing — Current situations and issues in selective system of public health nursing course at A University (in Japanese). *Tokyo Jikeikai Ika Daigaku Zasshi.* 2013; **128:** 99-107.

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Kubo T, Takahashi M, Ryu S, Togo F, Tanaka K, Shimazu A, Kubo Y, Kamata N, Uesugi J. Worker's fatigue and sleep coupled with changes in work-time control: a 1-year observational study (in Japanese). *Rodo Anzen Eisei Sogo Kenkyusho Tokubetsu Kenkyu Hokoku.* 2013; **43:** 137-41.

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Home Care Nursing

Motoko Kita, *Professor*
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General Summary

In Home Care Nursing, the lecture-based "Introduction to Home Care Nursing" and practice-based "Methodology of Home Care Nursing" and "Clinical Practice in Home Care Nursing" have been provided since 2011 to effectively help students develop practical nursing care skills step by step with due consideration of the characteristics of home care nursing. This year, we conducted course evaluations together with research performed in accordance with each member of the teaching staff's topics of interest.

Research Activities

Evaluation of practice-based classes focusing on the home care nursing process according to course evaluations by students

In the practice-based "Methodology of Home Care Nursing" provided in the first semester of the third year, patient cases are introduced to have the students assess them, identify issues, develop nursing care plans, and perform role playing to improve their practical skills. To examine and improve the practice-based home care nursing course, we continuously evaluated the course. The students generally assessed the practice-based home care nursing course positively with regard to the adequacy of the number of classes and teaching materials, students' learning efforts, understanding of home care patients, planning, role playing, and the involvement of the teaching staff. As issues requiring attention, we observed the following: 1) support for group work, 2) improving the lesson structure and training system for role playing, and 3) improving the planning support in accordance with the characteristics of each group.

Evaluation of practice-based classes focusing on the home care nursing process: influence on the students' level of goal attainment in "Clinical Practice in Home Care Nursing"

In this study, students' self-assessments after the practice-based classes and after the subsequent practical training in "Clinical Practice in Home Care Nursing" were compared to assess the effect of the students' self-assessments after the classes on their level of goal attainment when completing "Clinical Practice in Home Care Nursing." We observed correlations between the degree of understanding of the nursing process and a higher level of goal attainment in "Clinical Practice in Home Care Nursing," suggesting the effectiveness of the existing series of learning processes for home care nursing.

A study of the development of the process to support elderly patients with dementia to return home following a stay in acute care hospitals
Recently, an increasing number of elderly patients with dementia are being admitted to acute care hospitals for the treatment of other diseases. However, it is difficult to effectively support their return home following the hospital stay. As the first step to develop a model to resolve the difficulty in supporting elderly patients with dementia to return home following a stay in an acute care hospital, we investigated the process involving nurses in charge of providing such support.

The effect of intervention with narrative approaches on the anticipatory grief of patients' families providing terminal care at home
To examine the effect of intervention with narrative approaches on the anticipatory grief of patients' families providing terminal care at home, we conducted continuous research using narrative approaches involving the families of patients with 6 months or less to live.

Basic research (Volume 2) on network development concerning the preventive long-term care support network
We believe that developing a network to identify patients in need of preventive long-term care through an effective use of regional resources in cooperation with regional groups, local volunteers, and various professionals is a new key to help solve issues concerning preventive long-term care. As basic research, we conduct interviews involving subjects, who belong to the staff of community general support centers, to assess the information, needs, and resources of regional groups with regard to preventive long-term care and categorize the data and examine issues in accordance with regional characteristics.

Inspection/evaluation
From the course evaluation performed this year, we observed the effectiveness of the existing series of learning processes for practice-based classes focusing on the home care nursing process. On the other hand, we have also observed issues, such as that the number of patient cases introduced in the practice-based classes and the class system may require reassessment due to the increasing number of students and that the lecture-based classes suffer because of the poor comprehension of students. To resolve these issues, it is necessary to further improve the classes and continue course evaluation.
The research performed by the teaching staff covers important topics in home care nursing. We hope to facilitate mutual support in the field and further develop our research.

Publications

Kita M, Ito K (Tokyo Women's Med Univ). The caregiving process of the family unit caring for a frail older family member at home: a grounded theory study. *Int J Older People Nurs.* 2013; **8:** 149-58.

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