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## Introduction

It is my great pleasure to publish *Research Activities 2011*, which is a report on the scientific and educational activities at The Jikei University School of Medicine in 2011. We publish a Japanese version each year in parallel with this English-language version. This issue contains the research activities in the departments, institutes, and laboratories of the Medical Science Center at The Jikei University School of Medicine in 2011. In this issue, only selected papers published by each department, institute, and laboratory are listed at the end of each report owing to limitations of space. Similarly, the names of staff are limited to assistant professor and above.

*Research Activities* is a short summary of the annual research works at The Jikei University School of Medicine. I hope that *Research Activities* is used by people outside our university as well as by the faculty members of our university. Like medical services, research and education are fundamental and important activities performed at the attached hospitals of a medical university.

The founder of The Jikei University School of Medicine, Baron Kanehiro Takaki, made great efforts to prevent beriberi, which was a serious disease among young people in the Meiji Period. He devised various diets that were intended to prevent beriberi. Kanehiro Takaki suggested that a constituent of food was important and that the ratio of nitrogen to carbon was critical. Independently from Takaki, Christiaan Eijkman, a Dutch hygienist, suggested that rice germ contained an anti-beriberi factor. His suggestion was developed to become the concept of new nutrient factors in food called vitamins. For his work, in 1929 Eijkman shared the Nobel Prize in Physiology or Medicine with Sir Frederick Hopkins, a British biochemist at Cambridge University. Takaki was not able to identify the factor that prevented beriberi, but he saved the lives of many Japanese Navy sailors by adding nitrogen, meat, and wheat to their diets. However, Takaki's suggestion that wheat was effective for preventing beriberi was not accepted in Japan, and many Japanese people continued to die of beriberi (later found to be caused by a deficiency of vitamin B<sub>1</sub>).

Takaki was not a basic scientist and was never able to identify the anti-beriberi factor in food. However, his suggestion that some anti-beriberi factor was present in wheat or meat was valued highly abroad but, unfortunately, not in Japan. Thus, Takaki's research is recognized as practical research. Research activities in both basic and clinical areas are fundamental for medicine and medical treatment.

I hope that all people working in clinical and basic science departments and the Research Center for Medical Sciences at our university will have research in mind and will accept the challenge of exploring the mechanisms of life and disease. I would be extremely pleased if the results of our research works contribute to the treatment of patients.

I greatly appreciate the cooperation of Professor Naofumi Kimura, Editor of the Jikeikai Medical Journal, and Professor Masao Okazaki in editing this report.

I am also grateful to the members of the Academic Information Center for their help in the preparation of this report.

Satoshi Kurihara  
President  
The Jikei University School of Medicine

November 15, 2012

## Continuing Medical Education Center

### The Continuing Medical Education Committee

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Toshiaki Abe, *Director*  
Yashuo Toriumi  
Katsuyoshi Tojo

Keizo Takagi  
Akihiko Ohno

#### General Summary

The Continuing Medical Education (CME) Center was established in 1982 to commemorate the centennial of The Jikei University and to support the education of doctors outside the university hospital. Registered members consist of alumni throughout Japan, members of the local medical association, and 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: 225 (as of April 1, 2012)  
Members using the Center: 127/year  
Telephone service: 71 cases
2. The 32nd summer seminar was held on August 6, 2011. Fifty-two persons participated.
3. Monthly seminars were held on the second Saturday afternoons of the month in April, May, June, July, September, November, and March. Twenty-five to 30 persons attended each seminar.
4. The "CME Center News" is mailed monthly to registered members.

## Center for Medical Education

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Osamu Fukushima, *Professor and Director*  
 Mariko Istubo, *Professor*  
 Toshikazu Sakuyama, *Associate Professor*  
 Masato Matsushima, *Associate Professor*  
 Nobuyuki Furutani, *Associate Professor*  
 Machiko Hirao, *Associate Professor*

Naofumi Kimura, *Professor*  
 Tetsuya Kawamura, *Associate Professor*  
 Hisashi Onoue, *Associate Professor*  
 Mariko Nakamura, *Associate Professor*  
 Hiroyuki Takahashi, *Associate Professor*  
 Yoshio Ishibashi, *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 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; technical support of faculty and management of faculty development and education seminars; and 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 Center now consists of the Office of Medical Education, the Office for Nurse Career Support, and the Office of Educational Development. The offices contribute to undergraduate educational activities in both the medical and nursing schools, mandatory postgraduate clinical training, staff development in the 4 attached hospitals, and management of e-learning systems and simulation training centers for students, faculty, staff in attached hospitals, and healthcare providers in the community.

### Research Activities

1. Our proposal, “Learning assessment system for advancing students’ growth,” was selected by MEXT to receive a Supporting Grant for Implementing University and Supporting Student Learning Program 2010. This project aims to establish computer-based testing for cumulative tests in the Schools of Medicine and Nursing and a portfolio evaluation system to ensure that each student is fit to practice as a healthcare provider upon graduation. Although the first trial of computer-based testing failed last year because of problems of the local network, this year we administered tests to students in years 2, 4, and 5 with an improved computer-based testing system. The portfolio system is also working well. Feedback data to students from out-of-campus institutions are converted to PDF files, which are collected in the portfolio saver and are used for nurturing students’ fitness to practice.
2. We promoted an Innovating University Education project supported by MEXT titled

“Research for Improvement in Medical and Dental Education.” In this project, we investigated quality assurance for medical education in the United Kingdom. We visited the General Medical Council, St. George Medical School, and the School of Medicine, King’s College. The General Medical Council requests self-evaluation forms to a school send external raters to the school, receives external evaluation forms written by external raters, and requests action plans to improve the school. This cycle is repeated every 5 years. Furthermore, these forms are made available on the Internet.

3. We promoted “Research on Nurse Practitioners Working with Cancer Specialists (physicians and pharmacists)” with a Supporting Grant for Clinical Cancer Research 2010 from the MHLW. We investigated Japanese physicians’ attitudes regarding the education of nurse practitioners by sending surveys to specialists (members of the Japan Society of Clinical Oncology) and generalists (members of the Japanese Primary Care Association and directors of clinical training programs for physicians).

4. We promoted “Historical Research on the Development of Medical Education in Japan” supported by a Grant-in-Aid for Scientific Research from MEXT. We investigated changes in medical education in Japan from 1945 to the present: reform by the General Headquarters of the Allied Forces just after the World War II, the introduction of the national licensure examination and postgraduate clinical training for interns, the creation of standards for establishing universities, changes in postgraduate clinical training, the proliferation of medical schools, implementation of the common achievement test system, and the introduction of institutional accreditation defined in the School Education Law. The research group published a book titled *History of Medical Education in Japan*.

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

6. Contribution to other institutions of higher education (faculty development lectures and workshops): Ryukyu University Hospital (May), Nihon University Hikarigaoka Hospital (June), Teacher Training of Judo Therapy Schools held by MHLW (June), Wakayama Prefectural Medical School (July), Showa University (July and November), Nara Prefectural Medical School (September), Hyogo Medical School (September), Saitama Medical School (October), Fukui University (October), Ibaragi Nursing School (October), Ryukyu University (November), Tokai University (November), Tokushima University (December), Kokusai Iryoufukushi University (December), Teacher Training of OT/PT Schools held by MHLW (January), Japanese School Association of Judo Therapy (January), Daitobunka University (February), and School of Dentistry, Iwate Medical University (February).

## Reviews and Books

**Fukushima O.** Patient safety education: Inter-Professional Workshop at Jikei (in Japanese). *Iryo no Shitsu Anzen Gakkaishi*. 2011; **6**: 371-3.

**Sakuyama T, Okazaki F, Nakamura M, Komatsu K, Shiobara K, Fukushima O.** Home

medical support at Jikei Medical University (in Japanese). *Gan to Kagaku Ryoho*. 2011; **38** Suppl.1: 29-33.

**Fukushima O, Ito K.** Preparation of student practice at the workplace and student evaluation

(in Japanese). *Rinsyo Hogaku Semina*. 2011; **10**: 22-39.

**Fukushima O.** Institutional reform on medical

education after the War II. In: Sakai T. History on Medical Education in Japan. Sendai: Tohoku University Press; 2012. p. 213-45.

## Department of Anatomy (Gross Anatomy and Neuroanatomy)

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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 organization of neuronal networks and their development are investigated to elucidate brain function and diseases using 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 explored by examining cadavers and animals.

### Research Activities

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

Region- and size-specific neuronal organizations of the caudal nucleus of the tractus solitarius (cNTS) were investigated, after which excitatory and inhibitory synaptic input patterns onto specific cell types were analyzed with 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 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 cells in this region. Small neurons ( $<150\ \mu\text{m}^2$ ) represented about 80% of the cell population of the cNTS. Predominant excitatory postsynaptic currents were observed in the adult small neurons, whereas inhibitory postsynaptic currents were more evident in larger neurons, irrespective of subnuclear location. This distinct differentiation of postsynaptic current patterns was not evident in neonates. GABAergic synapses were more frequently associated with dendrites of large catecholaminergic cells (73%) than with those of small calbindin-containing cells (10%) in adults. These results indicate that differential synaptic input patterns were developmentally established in distinct small and large neurons.

*Local axonal arborization patterns of distinct neuronal types in the cNTS*

Neurons in the cNTS are heterogeneous in cell size (50 to  $450\ \mu\text{m}^2$  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 bio-

cytin-filled cells after whole-cell patch-clamp recordings. On the basis of the patterns of axonal branching behaviors, cNTS cells could be classified into 2 groups: smaller cells (mean somal area,  $94.1 \mu\text{m}^2$ ; range,  $62\text{--}120 \mu\text{m}^2$ ;  $n=22$ ) and larger cells (mean somal area,  $245 \mu\text{m}^2$ ; range,  $142\text{--}411 \mu\text{m}^2$ ;  $n=23$ ). 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: 1) smaller cells had larger form factors than did larger cells ( $P<0.05$ ); and 2) larger neurons had more extensive dendritic arborization, expressed 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 the 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 immunocytochemically at both the light and electron microscopic levels. The counting 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 synapses 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 the 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 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 implicated in this process, and their blockade leads to disruption of normal circuit formation. This phenomenon has been thoroughly studied in several neural systems, including the somatosensory system, but has not yet been evidenced in the visceral sensory system. Ultrastruc-

tural 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 on 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 electrophysiology, into 2 cell groups: small 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 undifferentiated synaptic networks, possibly preventing synapse elimination and subsequent stabilization of the proper wiring.

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

Astrocytes are thought to be active participants in synaptic plasticity in the developing nervous system. Previous studies have suggested that the number of axosomatic synapses on the small cells of the rat cNTS decreases toward the end of the first postnatal week. Astrocytes might be involved in this phenomenon. We used light and electron microscopy to examine the morphological development of astrocytic processes around the small cell soma in the rat cNTS. Structures within the cNTS positive for glial fibrillary acidic protein, glutamate-aspartate transporter, and glutamate transporter 1 became more intensely stained as development proceeded. Structures positive for glutamate-aspartate transporter 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 participate in regulating the decrease of 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 key roles in respiratory, cardiovascular, and gastrointestinal functions, contains GABAergic neurons to regulate neuronal firing. In the present study, GABAergic neuronal organization was analyzed in relation to the location of subnuclei in the mouse cNTS. According to the differential expression of glutamate decarboxylase 67 (GAD67), vesicular glutamate transporter 2 (VGLUT2), calbindin, and tyrosine hydroxylase messenger RNAs, the cNTS was divided into 4 subnuclei: the subpostrema, dorsomedial, commissural, and medial subnuclei. The numerical density and size of soma in the 4 subnuclei were then quantified through analysis of an unbiased dissector. Calbindin-positive cells constituted subpopulations of small non-GABAergic neurons preferentially localized in the subpostrema subnucleus. Tyrosine hydroxylase-positive cells constituted large neurons preferentially localized in the medial subnucleus. GABAergic neurons constituted a subpopulation of small neurons, prefer-

entially localized in the commissural and medial subnuclei, which represented  $\geq 50\%$  of small cells in these subnuclei. Thus, the small GABAergic neurons were located around large tyrosine hydroxylase-positive 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 small GABAergic 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 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 dorsal subnuclei, the percentage of glutamate decarboxylase-positive terminals on the somata, the percentage of small cell somata with synapses, and the axosomatic synapse density markedly decreased from P5 to P10. In ventral subnuclei, the percentage of glutamate decarboxylase-positive terminals on the soma, the percentage of small or large cell somata with synapses, and the axosomatic synapse density were maintained or increased from P5 to P10. Thus, a decrease in inhibitory axosomatic synapses in dorsal subnuclei might facilitate maturation of fine receptive areas for peripheral inputs, whereas an increase in inhibitory axosomatic synapses in 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 the 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. The morphologic and chemical features of NTS neurons displayed 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 presynaptic and postsynaptic axodendritic arbor overlap of reconstructed neurons (according to parent somal distance) confirmed a heterogeneity of microcircuit connectivity that might 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 and suggested 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 subpopu-

lation 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.

### **Publications**

**Negishi Y, Kawai Y.** Geometric and functional architecture of visceral sensory microcircuitry. *Brain Struct Funct.* 2011; **216**: 17-30.

**Hashimoto T, Ojiri H, Kawai Y.** The foramen of

Huschke: age and gender specific features after childhood. *Int J Oral Maxillofac Surg.* 2011; **40**: 743-6.

## Department of Anatomy (Histology and Embryology)

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Masataka Okabe, *Professor*  
Toshiaki Tachibana, *Assistant Professor*  
Yasuyo Shigetani, *Assistant Professor*

Hisashi Hashimoto, *Professor*  
Hideaki Suzuki, *Assistant Professor*

### General Summary

Our group is interested in the developmental and evolutionary aspects of human organs. By comparing organ development in humans and other 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

#### *Temporal and spatial cellular distribution of neural crest derivatives and alpha cells during islet development*

Recent studies have shown that signals from neural crest (NC) derivatives regulate the mass, proliferation, and maturation of beta cells in the developing fetal pancreas. However, little is known about the cellular distribution of NC derivatives during pancreatic development or the process whereby the developing islets are enclosed. We studied the temporal and spatial distribution of NC derivatives and endocrine cells at each developmental stage. At embryonic day 10.5 (E10.5) of the mouse embryo, NC derivatives that migrated to the prospective pancreatic region were distributed near pancreatic epithelial cells. As development proceeded, most NC derivatives surrounded endocrine rather than exocrine cells and were distributed nearer to alpha cells than to beta cells. At E20, approximately 70% of the NC derivatives enclosing endocrine cells were distributed near alpha cells. Moreover, the expression of synaptic cell adhesion molecule (SynCAM), a  $\text{Ca}^{2+}$ -independent homophilic transcell adhesion molecule, was confirmed from E16.5 and was more prevalent at the cell boundaries of alpha cells and NC derivatives. These findings suggest that NC derivatives are distributed near alpha cells as a result of homophilic binding of SynCAM expressed by alpha cells and NC derivatives during islet development.

#### *Molecular biological and histopathological analysis of the novel ataxia mouse*

In the progressive ataxic gait mouse (the ataxic mouse), which was developed in our laboratory, a difficulty in exercising the hind limbs manifests at about 4 weeks of age and becomes more severe with age. This disorder is inherited in an autosomal recessive manner, but the responsible gene has not been identified. A linkage analysis of this disorder and single nucleotide polymorphisms (SNPs) in crossbreds of the ataxic Institute for Cancer Research (ICR) line and the C57BL/6J strain revealed a SNP that was conclusively linked to the onset of this disorder. The SNP was located on chromosome 2, and

the causative gene for this disorder was estimated to be localized in a gene segment of 10 million base pairs.

Neuropathological changes in the cranial nerves of the ataxic mouse were investigated. Many vacuoles were found in the trigeminal nerve, the facial nerve running in the temporal bone, and in the trigeminal ganglion. These vacuoles showed intense immunoreactions for neurofilaments, parvalbumin, and calbindin. However, symptoms caused by this vacuolar degeneration of neurons have not been clarified.

*The development of a novel transgenic mouse for in-vivo ribosome profiling*

Cardiomyocytes comprise at least 3 types of cell with different origins: the first heart-field origin, the second heart-field origin, and the proepicardium origin. Each type of cell might have a different gene regulatory network. To clarify differences in physiologic function by analyzing the comprehensive and cell-type-specific gene regulatory network, we plan to develop a new transgenic mouse in which ribosomal protein RPL10a might be expressed with either Halo7-tag or 3xFlag-tag in a cell-type-specific manner. The tagged RPL10a might be able to purify the ribosome with messenger RNAs under translation.

First, we constructed cytomegalovirus-promoter vectors expressing each tagged RPL10a and examined the intracellular localization of the tagged proteins in HEK293 cells. As expected, both of the tagged proteins localized at ribosomes and rough endoplasmic reticulum. Next, we constructed a vector expressing the tagged proteins exchanged in a Cre-recombinase-dependent manner. In expression experiments, the 3xFlag-RPL10a proteins were exchanged for the Halo7-RPL10a proteins when Cre-recombinase was co-expressed.

To express the tagged RPL10a specifically in striated muscles, we examined the function of the myosin heavy chain 6 (Myh6) promoter. The 5.8 kb upstream from the start codon of Myh6 was inserted into the luciferase-reporter vector by means of the Red/ET system. Promoter activity was measured with luciferase activity in C2C12 cells. The luciferase activity was weak in the logarithmic growth phase but strongly increased after induction of differentiation in a serum-starvation culture in 0.5% serum for 6 days. To complete the transgenic vector, the cytomegalovirus promoter of the tag-exchange vector replaced the Myh6 promoter.

Furthermore, we established MCF7 cell lines stably expressing Halo7-RPL10a to examine experimental conditions for performing ribosomal profiling. Using these cell lines, we will examine a ribosomal extraction method, a nuclease treatment method, a subtraction method for ribosomal RNA removal, a library-producing method for next-generation sequencing and a data-treatment method. In the future, we plan to develop a transgenic mouse.

*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—the 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 to find that the additive effects of BMP4 and fibroblast growth factor (FGF) 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 the future epidermis. We then examined the effect of *Dlx5* downstream genes that are expressed in the neural plate and its border region on the induced epithelium. 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 neural plate marker *Sox2*. The preplacodal ectoderm or ridge (PPE or PPR), which was recently reported by Streit et al and other groups, 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, which gives rise to the hypophyseal, nasal, lens, trigeminal, otic, and epibranchial placodes 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 were also increased, albeit only slightly. A heterogeneity test with an antibody against *Dlx5* on the induced epithelium showed that it uniformly expressed *Dlx5*. This study thus suggests that 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.

#### *The study of the diaphragm: Development and acquisition*

The diaphragm is a muscular membrane that developed only in mammals. It separates the body cavity into the thoracic cavity and the abdominal cavity and has important roles in respiration. The development of the diaphragm is not completely understood, but research on diaphragm development would be useful for elucidating the pathogenesis of congenital diaphragmatic hernia.

We used in situ hybridization to compare the genes responsible for diaphragmatic development in the mouse, which has a diaphragm, and the chick, which does not. We found that *Sim2* was important in the muscle differentiation of the diaphragm and was not expressed in the chick embryo. This finding suggests that *Sim2* is the key molecule for acquisition of the diaphragm in mammals.

#### **Publications**

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**Katsu K, Tokumori D, Tatsumi N, Suzuki A, Yokouchi Y.** BMP inhibition by DAN in Hensen's node is a critical step for the establishment of left-right asymmetry in the chick embryo. *Dev Biol.* 2012; **363**: 15-26.

## Department of Molecular Physiology

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Shigeru Takemori, *Professor*

Maki Yamaguchi, *Assistant Professor*

### General Summary

Our efforts have been concentrated on elucidating the molecular mechanism to achieve biological function through the cooperative interaction of water and proteins.

### Research Activity

#### *The transverse relaxation process of protons in cartilages extracted from knee joints*

Osteoarthritis (OA) is a common disease that affects the majority of persons 60 years or older. Because the knee pain caused by OA decreases quality of life, a method for detecting OA at an early stage has been hoped for in recent years.

Therefore, we reevaluated nuclear magnetic resonance (NMR) images of knee joint cartilages obtained from healthy volunteers on the basis of the water state of cartilages extracted from a pig.

The transverse relaxation process of protons in the extracted knee joint cartilages was classified into 3 or 4 groups on the basis of the characteristic transverse relaxation time constant ( $T_2$ ) obtained with NMR. Both in human cartilages and in the extracted pig cartilages, the mobility of the water protons in the knee joint cartilages gradually decreased from the cartilage surface towards the bone surface. Analysis of the transverse relaxation process showed that most of the water in knee cartilage corresponds to the water in the reversed micelle with a diameter of a few angstroms. These results suggest a method can be developed to detect OA at an early stage.

#### *Phase-transition measurement of water components in skinned skeletal muscle*

Our previous studies of water in skeletal muscle sarcomeres found at least 5 water components distinguished by characteristic spin-spin relaxation rates. The overall interaction between water molecules and structural macromolecules has been shown to restrict water activity to differentiate these water components. However, details of the interaction remain unclear. To reveal details of the interaction, it is necessary to perform measurements that directly reflect molecular interactions. We performed phase-transition measurements with differential scanning calorimetry. During the melting process of a frozen skinned fiber of frog skeletal muscle, heat liberation occurred at several temperatures. This finding clearly indicates that the phase transition of each water component reflects its enthalpic state and that the temperature of the phase transition is a key to resolving the energetic profiles of interactions between water and structural macromolecules.

#### *Restoration of stable liquid crystalline structure of sarcomere*

The demembration procedure for preparing skinned skeletal muscle fibers destabilizes

the structure of sarcomeres. In our previous NMR study, we found that the diffusion of sarcomere macromolecules is responsible for this destabilization. On the basis of this understanding, we evaluated the X-ray diffraction patterns of muscle fibers to search for a macromolecule that would effectively restore the sarcomere structure. Among the macromolecules studied, polyethylene glycol of molecular weight 3350 (PEG 3350) was found to restore the lattice spacing of thick filaments to the physiological level. Combined with an agent, 2,3-butanedione monoxime, which stabilizes myoproteins in the resting state, PEG 3350 was successfully used to restore the stability of the sarcomere structure in skinned muscle specimens.

*Viscoelastic change of the myosin solution evaluated with a quartz crystal microbalance*

We observed the adsorption process of myosin to the surface of gold by means of a quartz crystal microbalance (AFFINIX QN Pro, Initium, Inc., Tokyo). The viscoelasticity of myosin and its surrounding solution adsorbed to the surface of a quartz crystal was estimated from the resonance frequency (reflecting the weight of adsorbed myosin) and the width of the spectrum (reflecting the change in the viscoelasticity of myosin and the surrounding solution).

When the density of myosin adsorption was less than  $0.2 \mu\text{g}/\text{cm}^2$ , the viscoelasticity was almost the same as that of the buffer without myosin. This finding suggests that myosin adsorbed at a low density acts as a solid globular protein. On the other hand, when myosin is adsorbed at a higher density, the large viscoelastic change observed indicates that myosin acts as a protein having a significant numbers of bound water molecules nearby.

## Publications

**Watanabe Y<sup>1</sup>, Tatsumi N<sup>2</sup>, Takemori S** (<sup>1</sup>*Seijo Univ*, <sup>2</sup>*Ibaraki Univ*). Easy evaluation of the risk of heat disorders during midsummer kendo practice (in Japanese). *Budogaku Kenkyu*. 2011; **44**: 1-12.

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<sup>2</sup>*Ryutsu Keizai Univ*, <sup>3</sup>*Seijo Univ*, <sup>4</sup>*Hokkaido Kyoiku Univ*). An evaluation of Kendo techniques using 3-Axis accelerometers (in Japanese). *Seijo Daigaku Keizai Kenkyu*. 2012; **195**: 33-46.

**Tanaka Y<sup>1</sup>, Watanabe Y<sup>1</sup>, Takemori S** (<sup>1</sup>*Seijo Univ*). Accelerometric evaluation of limb movements of badminton players: in search of efficient aid for skill advancement (in Japanese). *Seijo Daigaku Keizai Kenkyu*. 2012; **195**: 47-74.

## Department of Cell Physiology

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Satoshi Kurihara, *Professor*  
 Masato Konishi, *Visiting Professor*  
 Yoichiro Kusakari, *Assistant Professor*

Iwao Ohtsuki, *Visiting Professor*  
 Norio Fukuda, *Associate Professor*

### General Summary

The main research interest of our department is the physiology of cardiac muscle contraction.

### Research Activities

*Depressed Frank-Starling mechanism in left ventricular muscle of the knock-in mouse model of dilated cardiomyopathy with troponin T deletion mutation  $\Delta K210$*

We have demonstrated that the Frank-Starling mechanism is coordinately regulated in cardiac muscle via thin-filament “on-off” switching and titin-based changes in interfilament lattice spacing. In the present study, 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 a deletion mutation  $\Delta K210$  in the cardiac troponin T gene. Confocal imaging revealed that the cardiomyocytes were significantly enlarged, especially in the longitudinal direction, in the hearts of  $\Delta K210$  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. For analysis of the sarcomere length-dependence of active force, skinned muscles were prepared from the left ventricles of wild-type and  $\Delta K210$  mice. An increase in sarcomere length from 1.9 to 2.2  $\mu\text{m}$  shifted the midpoint ( $\text{pCa}_{50}$ ) of the force-pCa curve leftward by 0.21 pCa units in wild-type preparations. In  $\Delta K210$  muscles,  $\text{Ca}^{2+}$  sensitivity was lower by 0.37 pCa units, and the sarcomere length-dependent shift of  $\text{pCa}_{50}$ , i.e.,  $\Delta\text{pCa}_{50}$ , was less pronounced (0.11 pCa units), with and without treatment with protein kinase A. The rate of active force redevelopment was lower in  $\Delta K210$  preparations than in wild-type preparations, showing blunted thin-filament cooperative activation. An increase in thin-filament cooperative activation upon an increase in the fraction of strongly bound cross-bridges by MgADP increased  $\Delta\text{pCa}_{50}$  to 0.21 pCa units. We therefore conclude that the depressed Frank-Starling mechanism in the hearts of  $\Delta K210$  knock-in mice is the result of a reduction in thin-filament cooperative activation.

*Real-time measurement of sarcomere length in the mouse heart in vivo by means of  $\alpha$ -actinin-green fluorescent protein*

Despite numerous studies performed under various experimental settings, the molecular mechanisms of contraction and relaxation of cardiomyocytes remain elusive *in vivo*. In the present study, we expressed green fluorescent protein (GFP) at sarcomeric Z-disks ( $\alpha$ -actinin) by means of an adenovirus vector system in adult mice and performed real-

time imaging of the movement of single sarcomeres in cardiomyocytes in the left ventricle under fluorescence microscopy at 10-nm precision (at 100 fps). First, we found that sarcomere length was 2.0  $\mu\text{m}$  in the isolated heart when perfused with 30 mM 2,3-butanedione monoxime (hence, at rest). This value is close to values previously obtained in rats by other investigators with various experimental techniques. When perfused with Tyrode's solution containing 1 mM  $\text{Ca}^{2+}$ , the heart started to beat with diastolic and systolic sarcomere lengths of 2.2 and 1.7  $\mu\text{m}$ , respectively, in the left ventricle. Finally, we attempted to visualize single sarcomeres *in vivo* in open-chest mice under anesthesia. We found that sarcomere length was 2.0 and 1.7  $\mu\text{m}$  during diastole and systole, respectively, but varied by 0.3  $\mu\text{m}$  even in the same left ventricular cell. We also found that sarcomere contraction occurred at the T-wave endpoint on electrocardiograms, followed by an increase in left ventricular pressure.

#### *Microscopic analysis of spontaneous sarcomeric oscillations in neonatal cardiomyocytes*

We have previously demonstrated that cardiac myocytes shows rhythmic, spontaneous sarcomeric oscillations (SPOCs) under partial activation states, namely, at  $\text{pCa}=6.0$  (Ca-SPOC) or in the presence of MgADP and Pi under relaxing conditions (ADP-SPOC). We have reported that the period of SPOC (both Ca-SPOC and ADP-SPOC) in skinned myocardium correlates with the period of the resting heart rate in various animal species. To fully understand the molecular mechanisms of SPOC, in the present study we used neonatal rat cardiomyocytes expressing  $\alpha$ -actinin-GFP and visualized the motions of sarcomeres. First, we successfully induced SPOCs in neonatal myocytes with an intact inner-membrane system following treatment with ionomycin by controlling cytoplasmic  $\text{Ca}^{2+}$  concentrations. We have termed this phenomenon Cell-SPOC. The measurement of intracellular  $\text{Ca}^{2+}$  with fluo-4 confirmed that  $\text{Ca}^{2+}$  oscillations did not occur under our experimental conditions. As found in adult cardiomyocytes, the sarcomeric oscillations consisted of quick lengthening and slow shortening during Cell-SPOC. We also found in untreated neonatal myocytes that an increase in the frequency of electrical stimuli to the physiological level (i.e., 3 to 5 Hz) caused a phase shift of shortening and relengthening due to enhancement of the relengthening speed, resulting in a waveform similar to that observed during Cell-SPOC in ionomycin-treated cardiomyocytes. These results suggest that the auto-oscillatory properties of cardiac sarcomeres are involved in the regulation of heartbeat.

#### *$\text{Ca}^{2+}$ -independent on-off regulation of cardiomyocytes by microscopic heat pulses*

Laser irradiation is a novel technique of noninvasive stimulation in cardiac and neural tissues. However, physical parameters for the laser irradiation-induced cardiac contractions have not been clarified, because various physicochemical reactions, such as photochemical and photothermal effects, are triggered in this process. Here we studied the effects of laser-induced local temperature changes on the functions of isolated cardiomyocytes. We have demonstrated previously that a microscopic heat pulse ( $\Delta T=0.2^\circ\text{C}$  for 2 seconds) induces a  $\text{Ca}^{2+}$  burst in cancer cells (HeLa cells) at body temperature, with a mechanism similar to that of rapid cooling contracture in skeletal and cardiac muscles. In the present study, we generated microscopic heat pulses by focusing infrared

laser light in the extracellular solution near adult rat cardiomyocytes. We found that a microscopic heat pulse ( $\Delta T=5^{\circ}\text{C}$  for 0.5 second) induces contractions at a basal temperature of  $36^{\circ}\text{C}$ . At  $25^{\circ}\text{C}$ , a larger  $\Delta T$  was required to induce contractions. When 2.5-Hz heat pulses were repeatedly applied, we observed oscillatory contractions of cardiomyocytes. Unlike contractions induced by electric stimulation, these contractions were not accompanied by  $\text{Ca}^{2+}$  transients. Likewise, heat pulses induced contractions of skinned cardiomyocytes in a  $\text{Ca}^{2+}$ -free solution in the presence of ATP. These results demonstrate that heat pulses can regulate cardiac contractions without any involvement of  $\text{Ca}^{2+}$  dynamics, by directly activating the actomyosin interaction. Hence, our microheating technique may be useful for stimulating the beating of failing hearts without causing abnormal  $\text{Ca}^{2+}$  dynamics.

#### *$\text{Ca}^{2+}$ handling and contraction in cardiac papillary muscles with interstitial 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  $\text{Ca}^{2+}$  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 weight of PAB rats was significantly greater than that of control rats, indicating right ventricular hypertrophy. Right ventricular papillary muscles of the PAB rats were divided into an interstitial fibrosis group and a nonfibrosis group by using Masson trichrome staining for comparison with those of the control group. To measure tension with intracellular  $\text{Ca}^{2+}$  transients, we used the aequorin method. The peak  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  in the interstitial fibrosis group was significantly longer than that in 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. Depressed tension development of myocardium with interstitial fibrosis is likely due to: 1) lowering of myofibrillar  $\text{Ca}^{2+}$  sensitivity, 2) decreases in  $\text{Ca}^{2+}$  release, and 3) asynchronous activation of cardiomyocytes via impaired cell-to-cell communication.

#### *Pathophysiologic study of the heart in collaboration with the Division of Cardiology, Department of Internal Medicine*

We have investigated the role of  $\text{Ca}^{2+}$  handling in cardiac contractility under physiological and pathophysiological conditions, such as cardiac hypertrophy and heart failure. In the present study, we investigated the significance of the renin-angiotensin system in the pathogenesis of DCM in mice with troponin T deletion mutation  $\Delta\text{K210}$ . We used an angiotensin-receptor blocker and a direct renin inhibitor to block the renin-angiotensin system *in vivo*. We found that both the angiotensin-receptor blocker and the direct renin inhibitor were effective for treating cardiac contractility in DCM mice, with, presumably, different mechanisms of action.

## Publications

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**Serizawa T, Terui T, Kagemoto T, Mizuno A, Shimozawa T<sup>1</sup>, Kobirumaki F, Ishiwata S<sup>2</sup>, Kurihara S, Fukuda N (1Tokyo Univ, 2Waseda Univ).** Real-time measurement of the length of a single sarcomere in rat ventricular myocytes: a novel analysis with quantum dots. *Am J Physiol Cell Physiol*. 2011; **301**: C 1116–27.

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## Reviews and Books

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**Higuchi S<sup>1</sup>, Tsukasaki Y<sup>1</sup>, Fukuda N, Kurihara S, Fujita H<sup>1</sup> (1Riken Quantitative Biol Ctr).** Thin filament-reconstituted skinned muscle fibers for the study of muscle physiology. *J Biomed Biotechnol*. 2011; **2011**: 486021.

## Department of Biochemistry

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Kiyoshi Ohkawa, *Professor*  
Tadashi Asakura, *Associate Professor*

Koji Takada, *Associate Professor*

### Research Activities

#### *Cancer research*

1. Glucose metabolism is another target for cancer chemoprevention. CD147 is an accessory subunit of heteromeric lactate transporters, monocarboxylate transporters (MCTs), known as the SLC16 family of solute transporters. The MCTs transport lactate across the plasma membrane, and the interaction of CD147 and MCT is required for expression of MCT activity and for the trafficking of MCT molecules to the plasma membrane. The pyruvate/lactate analog 3-bromopyruvate (3-BrPA) is a potent glycolytic inhibitor and candidate anticancer agent. Last year, 3-BrPA was shown, with the MCT1/small interfering RNA technique and a small molecular MCT1 inhibitor, to be transported into PC-3 cells through the CD147-MCT1 heteromeric lactate transporter complex and to promote cell death. The cytotoxic activity of 3-BrPA against several cancer cell lines is enhanced under hypoxic conditions, because the expression of CD147 and MCT1 is greater than under normoxic conditions. To confirm the molecular chaperon function of CD147, the interacting proteins were screened with the co-immunoprecipitation method. Results revealed the existence of novel endogenous CD147-associated proteins, matrix metalloproteinase (MMP) 3 and carbonic anhydrases (CA9 and CA12), in addition to previously identified proteins, such as MMP1, MCT1, MCT4, and PDZ and LIM domain (PDLIM) 7.

2. Targeted chemotherapy against CD147-expressing carcinoma cells.

We studied the effects of anti-CD147 antibody-labeled polymeric micelles (aCD147ab-micelles) encapsulating a conjugate of glutathione and doxorubicin (GSH-DXR) on the specific accumulation and cytotoxicity against CD147-expressing human carcinoma cells. After treatment with an aCD147ab-micelle encapsulating GSH-DXR, a specific accumulation and cytotoxicity was observed in CD147-expressing cells.

3. E-cadherin suppression in epoxomicin-resistant cells may be regulated by expression of zinc finger E-box-binding homeobox (ZEB) 1. Six cell lines resistant to epoxomicin were established. The epoxomicin-resistant cell lines are reliable tools for evaluating the effects of proteasome inhibitors in preclinical trials. Moreover, these cell lines may also be useful for clarifying mechanisms of resistance to proteasome inhibitors and examining a wide variety of proteasomal functions. In an epoxomicin-resistant human endometrial carcinoma cell line, Ishikawa variant, E-cadherin gene (CDH1) expression was suppressed *via* overexpression of ZEB1, a transcriptional repressor of E-cadherin. Treatment of parental Ishikawa cells with epoxomicin immediately induced ZEB1, followed by transient suppression of E-cadherin expression. Expression of ZEB1 was followed by suppression of miR200 in epoxomicin-resistant endometrial carcinoma Ishikawa (Ish/EXM) cells, and expression of miR200 in Ish/EXM cells by means of

transfection of pre-miR200 repressed ZEB1 expression and recovered expression of E-cadherin. These results confirm that suppression of E-cadherin expression via ZEB1 expression is regulated by miR200 in Ish/EXM cells.

4. Targeting of the glycolytic pathway has become an attractive strategy for developing new anticancer agents. The pyruvate/lactate analog 3-BrPA is a potent glycolytic inhibitor. We have shown that 3-BrPA is transported into PC3 prostate carcinoma cells through MCT1 and promotes cell death. Then, we studied the cytotoxicity of 3-BrPA and protein expression of MCT1 using 20 different cell lines. The results suggested that 3-BrPA-sensitive cell lines tend to highly express MCT1 protein. Knockdown of MCT1 expression allowed several cell lines to survive despite treatment with 3-BrPA. On the other hand, MCT1 was expressed at low levels in the breast cancer cell line MDA-MB-231, which is resistant to 3-BrPA. We hypothesized that epigenetic silencing of MCT1 gene expression occurs in MDA-MB-231 cells. We found that a combination treatment of a DNA methyltransferase inhibitor (5-aza-2'-deoxycytidine) and a corticosteroid (dexamethasone) significantly increased MCT1 mRNA expression in MDA-MB-231 cells. Thus, gene silencing of MCT1 in 3-BrPA-resistant cells is likely regulated through DNA methylation.

#### *Other research*

The following 3 studies were performed: 1) toxicity evaluation of chemicals by quantification of cellular polyubiquitin chains, 2) study of production of plasma proteins by well-differentiated human hepatoma cell lines, and 3) biochemical study of ubiquitin-specific peptidase 46 (USP46) underlying despair behavior in mice.

1. To find novel markers for assessing the risk of chemicals, we quantified cellular polyubiquitin chain levels in proximal tubular epithelial HK-2, neuroblastoma Neuro2A, and fibroblast NIH/3T3 cells exposed to CdCl<sub>2</sub>, methyl mercury, or *N,N'*-dimethyl-4,4'-bipyridinium dichloride (Paraquat). We found that Cd exposure induced a marked increase in polyubiquitin chains in all cells and, therefore, might be useful as a toxicity marker.

2. To develop plasma protein derivatives having no infectious risks, we tested human hepatocellular carcinoma FLC-4 and FLC-7 cells and found optimal culture conditions for the production of albumin and fibrinogen.

3. Deubiquitinating activity was measured in brain tissues of the CS mouse, which have lost despair behavior due to a gene mutation of USP46, and the activity was found to be reduced in the hippocampus and olfactory bulb of CS mice in comparison with that controls. To construct a measurement system for wild-type and mutant USP46 activity, we prepared an expression system using episomal vector in mammalian cells. The USP46 proteins (wild-type and mutant) were stably overexpressed in HeLa cells.

#### **Publications**

**Iida Y, Aoki K, Asakura T, Ueda K, Yanaihara N, Takakura S, Yamada K, Okamoto A, Tanaka T, Ohkawa K.** Hypoxia promotes glycogen synthesis and accumulation in human ovarian clear

cell carcinoma. *Int J Oncol.* 2012; **40**: 2122-30. Epub 2012 Mar 19.

**Marushima H, Shibata S, Asakura T, Matsuura T, Maehashi H, Ishii Y, Eda H, Aoki K, Iida Y,**

**Morikawa T, Ohkawa K.** Three dimensional culture promotes reconstitution of the tumor specific hypoxic microenvironment under TGFbeta stimulation. *Int J Oncol.* 2011; **39**: 1327-36.

**Shimada Y, Kobayashi H, Kawagoe S, Aoki K, Kaneshiro E, Shimizu H, Eto Y, Ida H, Ohashi T.** Endoplasmic reticulum stress induces autophagy through activation of p38 MAPK in fibroblasts from Pompe disease patients carrying c.546G>T mutation. *Mol Genet Metab.* 2011; **104**: 566-73.

**Conlon JM, Mechkarska M, Ahmed E, Lep-rince J, Vaudry H, King JD, Takada K.** Purifi-

cation and properties of antimicrobial peptides from skin secretions of the Eritrea clawed frog *Xenopus clivii* (Pipidae). *Comp Biochem Physiol C Toxicol Pharmacol.* 2011; **153**: 350-4.

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## Department of Molecular Biology

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

Akihiro Oguro, *Assistant Professor*

### General Summary

Polyamines (putrescine, spermidine, and spermine) are ubiquitous biogenic amines that bind to nucleic acids and are essential for proliferation. Cellular polyamine contents are maintained by a feedback mechanism involving the key regulatory proteins antizymes (AZs). AZs are expressed by translational frameshifting, which is induced by polyamines, and negatively regulate cellular polyamines. In mammals there are 3 AZ isoforms (AZ1-3). AZs are further regulated by proteins termed AZ inhibitors (Azins). Cancer cells generally contain elevated levels of polyamines. Our goal is to clarify the mechanism and biological significance of the elaborate regulatory system and to develop polyamine-related research and diagnostic tools.

### Research Activities

#### *Role of AZ2 in c-Myc degradation under hypoxic conditions*

AZ binds to ornithine decarboxylase (ODC), a key enzyme for polyamine biosynthesis, to trigger degradation of ODC by the 26S proteasome. AZ1 reportedly interacts with several proteins other than ODC and accelerates their degradation. We have previously found in cultured cells that AZ2 accelerates c-Myc degradation by proteasomes and that this pathway is involved in c-Myc decay after UV irradiation. This year we showed via knockdown of AZ2 that the proteasomal degradation of c-Myc under hypoxic conditions is also mediated by AZ2. Hypoxia has been reported to cause both c-Myc degradation and an increase in cellular polyamines. Thus, our results suggest a novel pathway of hypoxia-induced c-Myc degradation mediated by polyamines and AZ2 in a ubiquitin-independent manner.

#### *Fluorescent visualization of cancer cells by monitoring of cellular polyamines*

We are developing a novel method to visualize cancer cells by combining the polyamine-dependent frameshift mechanism of AZ, an endogenous cellular polyamine sensor, and fluorescent protein techniques. This year we improved the reporter construct by using the entire protein coding region of human AZ1 messenger (m) RNA with the enhanced green fluorescent protein (EGFP) gene that is inserted immediately downstream of the pseudoknot structure. Cells transfected with the construct showed increases in both EGFP fluorescence and the frameshift product in response to the addition of polyamines to the culture medium.

#### *The effect of polyamines on hematopoiesis in adult mice*

We have previously shown in AZ1 knockout mouse embryos that elevated tissue poly-

amine levels disturb early stage hematopoietic cell differentiation in the liver, particularly production of the multipotent hematopoietic progenitor cells (MPPs). To determine whether polyamines also affect hematopoietic cell differentiation in the bone marrow of adult mice, we fed a high-polyamine diet to adult mice and analyzed bone marrow cells. We found that the high-polyamine diet decreased the number of MPPs in adult bone marrow. Polyamines accumulate in patients with renal failure, and 10% to 15% of patients with chronic renal failure have anemia that does not respond to treatment with erythropoietin. Thus, accumulation of polyamines might be a cause of erythropoietin-resistant anemia in renal failure.

#### *Multiple forms of mouse Azin1 differentially regulated by polyamines*

Homozygous *Azin1* gene trap mice show partial lethality with decreased tissue levels of putrescine, but low levels of *Azin1* mRNA and Azin1 protein are detected in their tissues, raising the possibility that alternative forms of *Azin1* mRNA are transcribed to skip the trapping insertion. Last year, we found various splicing forms of *Azin1* mRNA. This year, we found 5 new alternative transcriptional start sites (TSS1-5) downstream of the trapping insertion site. TSSs1-3 are located upstream of the authentic starting codon and would explain the presence of the full-length Azin1 in the mutant mice. TSS4 is located on intron 4, and the corresponding transcript encodes an N-terminal-truncated form of Azin1 (Azin1 $\Delta$ N). We also identified a splice variant with an extended exon 7 to the 5' direction which encodes a C-terminal-truncated form by a premature termination codon (Azin1 $\Delta$ C). Both Azin1 $\Delta$ N and Azin1 $\Delta$ C retained antizyme-binding activity. Interestingly, the levels of transcripts for the full-length Azin and Azin1 $\Delta$ C were reciprocally regulated by polyamines. These results suggest that polyamine-regulated splicing regulates Azin1 function by producing an alternative form of Azin1.

#### *Analyses of the spermine-binding site on RNA aptamer*

The technique of SELEX (systematic evolution of ligands by exponential enrichment) has been used to isolate high-affinity oligoribonucleotides called aptamers from randomized RNA libraries. Selected 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. The antispermine aptamer is predicted to contain 2 stem-loop structures (5' stem-loop and 3' stem-loop). Mutational analyses revealed that the 3' stem-loop bound spermine more effectively than did the 5' stem-loop. We identified a bulged (unpaired) structure in the 3' stem-loop (C/ACA) important for spermine binding, because a mutant without the bulge had markedly reduced binding activity. Further mutation analyses revealed that the A-U base-pair neighboring the bulged structure was also important for spermine binding. Moreover, <sup>1</sup>H-NMR (nuclear magnetic resonance) spectrum analysis revealed the G-U wobble base-pair next to the A-U pair is also concerned with spermine binding. These results suggest that this stem and bulge region is a hot spot for spermine binding.

*Analysis of molecular mechanism of carcinogenesis in ovarian clear cell carcinoma*

Amplification of chromosome 17q21-24 has frequently been observed in ovarian clear cell carcinoma (CCC). However, the driver gene of the region has not been identified. Aberrant expression of microRNAs has been shown to be involved in oncogenesis. MicroRNA-21 (miR-21) encoded on 17q21-24 is a frequently overexpressed microRNA in many types of cancer. On the basis of the above, we hypothesized that miR-21 plays important roles in CCC oncogenesis through the regulation of PTEN (phosphatase and tensin homologue) expression. Analysis of clinical samples revealed overexpression of miR-21 and repression of PTEN in cases of CCC with amplification of 17q21-q24.

## Department of Pharmacology

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Toshihiko Momiyama, *Professor*  
 Yuji Ohno, *Assistant Professor*  
 Taro Ishikawa, *Assistant Professor*

Naofumi Kimura, *Professor*  
 Haruhisa Nishi, *Assistant Professor*

### General Summary

The research interests of the Department of Pharmacology include:

- 1) Synaptic transmission and its modulation in the basal ganglia and basal forebrain (Toshihiko Momiyama)
- 2) Neural control of breathing in aquatic vertebrates (Naofumi Kimura)
- 3) Design of secretory proteins (Yuji Ohno)
- 4) i) Study of the mechanisms involved in the modification of allergic degranulation via purinergic receptors in a cell line derived from human mast cells (Haruhisa Nishi)  
 ii) Study of glucocorticoid production by activation of purinergic receptors in a human-derived adrenocortical cell line (Haruhisa Nishi)
- 5) Firing patterns of pontine neurons in cerebrocerebellar interaction (Taro Ishikawa)
- 6) The basic mechanism of a ketogenic diet: purinergic autocrine regulation of CA3 pyramidal neurons (Masahito Kawamura)
- 7) Visual response in the cerebellar parafoveolus (Misa Shimuta)

### Research Activities

#### *Synaptic transmission and its modulation in the basal ganglia and basal forebrain*

Electrophysiological studies using slice patch-clamp recording were performed to analyze synaptic transmission, its modulation by neuromodulators, and their developmental changes in the nigrostriatal and mesolimbic dopaminergic systems and the cholinergic system of the basal forebrain. These systems are involved in various psychological functions and their disorders, including Parkinson's disease and Alzheimer's disease. Electrochemical analyses with carbon nanotube, a new biosensor material, have also been performed to clarify the mechanisms of catecholamine release in the midbrain. Another issue is the regeneration of synapses and local circuits after basal-ganglia-related disorders. In these studies, electrophysiological, morphological, and behavioural studies were performed to clarify the mechanisms and time course of the reconstruction of synaptic organization, transmission, and functions of whole animals in Parkinson's disease model rats or cerebral ischemia model rats. In addition, the role of the phosphatidylinositol system in basal ganglia synaptic transmission was analyzed.

These basic analyses could lead to the identification of the mechanisms underlying the related disorders mentioned above and to the development of novel therapeutic tools.

#### *Neural control of breathing in aquatic vertebrates*

Yawning is a common behavioral event in almost all vertebrates. In this study a video

camera was used to investigate yawning in amniotes and nonamniotes. Yawning in turtles (which originated later than in mammals) and in vertebrates more basal than mammals (amphibians, lungfish, ray-finned fishes, and sharks) consisted of maximal opening of the mouth and lowering of the oropharyngeal (buccal) floor. Interestingly, aquatic turtles, amphibians, and air-breathing fishes (lungfish, gar, and polypterus) never open their glottis during yawning, unlike mammals. These observations indicate that yawning is a common behavior in jawed vertebrates and is more primitive than lung ventilation. Opening of the glottis would be a characteristic for yawning in some specific vertebrates, such as mammals. A possible reason mammals open their glottis during yawning is that the ancestral mammalian used part of the nerve activity that lowers the buccal floor to drive the diaphragm in the evolutionary process.

#### *Design of secretory proteins*

We found that almost all mouse interleukin (IL) 31 is secreted from human embryonic kidney cells when the protein is obligatorily expressed in cells transfected with a mammalian expression plasmid and a cytomegalovirus promoter. We then confirmed that the fusion protein of enhanced green fluorescent protein and the cytokine is also efficiently secreted. As we investigated the secretory sequences, we hypothesized that the N-terminal sequences of IL-31 from signal peptides to the first glycosylation site (SG sequences) are crucial. Furthermore, we examined the fusion proteins of SG-sequences with p53, which has nuclear localization signal sequences, and aquaporine, which is a membrane protein. We were able to design several secretory proteins associated with SG sequences.

#### *Study of the mechanisms of the modification of allergic degranulation via purinergic receptors in a cell line derived from human mast cells*

The aims of the present study were to investigate the function and intracellular mechanisms of purinergic receptors in LAD2 human-derived mast cells and to determine the possibility of controlling degranulation via purinergic systems. The findings suggest that purinergic receptors modulate the intracellular enzymatic cascade between phosphatidylinositol-3 kinase and Akt, a Ser/Thr kinase, and have some effects on kinase activity downstream of E $\epsilon$ RI activation leading to enhancement or inhibition of Fc $\epsilon$ RI-induced allergic degranulation. The findings also suggest the possibility that controlling purinergic systems on mast cells is a new therapeutic approach for type I allergy.

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

The human adrenocortical cell line H295R was used to investigate the function of purinergic systems in human adrenocortical steroidogenesis. The results suggest that H295R cells express multiple and functional purinergic receptors for intracellular Ca<sup>2+</sup>-mobilization and that the P2Y<sub>1</sub> subtype of the purinergic receptor is linked to the store-operated Ca<sup>2+</sup> entry activation, leading to Ca<sup>2+</sup>-influx which might be necessary for glucocorticoid production. On the other hand, some purinergic receptors expressed on H295R cells were found to be linked to production of cyclic adenosine monophosphate. The present

findings suggest that some functional purinergic systems and the crosstalk of intracellular second messengers for steroidogenesis are present in human adrenal cortex.

#### *Firing patterns of pontine neurons in cerebrocerebellar interaction*

Somatosensory signals from the cerebral cortex of rodents are delivered to the cerebellum via the pontocerebellar pathway. Somatosensory stimulation to the whiskers and the perioral skin triggers high-frequency burst firing of the projecting mossy fibers. However, the cellular mechanisms that generate this high-frequency firing are not known. Therefore, we investigated firing properties and synaptic currents in projection neurons in the pontine nuclei in acute slice preparations and in vivo in anesthetized animals. The results indicate that the pontine nuclei neurons can fire at a high frequency by depolarization but that low-frequency synaptic inputs do not trigger firing. We are investigating the firing properties of pontine nuclei neurons in further detail.

#### *The basic mechanism of a ketogenic diet: Purinergic autocrine regulation of CA3 pyramidal neurons*

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. A ketogenic diet increases ATP concentrations in the central nervous system and causes mild hypoglycemia. To clarify the role of extracellular purines underlying the anticonvulsant effect of the ketogenic diet, whole-cell voltage clamp recordings were made from CA3 pyramidal neurons in acute hippocampal slices from rats. In conditions of reduced extracellular glucose and high intracellular ATP concentrations, CA3 pyramidal neurons hyperpolarize themselves via direct ATP release through pannexin-1 channels, with the subsequent activation of adenosine A<sub>1</sub> receptors. This autocrine regulation might be an important mechanism underlying the success of a ketogenic diet.

#### *Visual response in the cerebellar paraflocculus*

Our previous studies showed that the cerebellar paraflocculus receives visual signals and that most granule cells in this area respond to visual stimuli. However, responses of Purkinje cells in this area have not been investigated. Thus, we recorded from the Purkinje cells of anesthetized rats. The results indicated that the visual stimuli trigger a change in the frequency of “simple spikes” of the Purkinje cells but do not trigger “complex spikes.” We are now exploring stimulation methods that can evoke simple and complex spikes independently by using direct electrical stimulation of the cerebral cortex.

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## Department of Pathology

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Hiroshi Hano, *Professor*  
Akihiko Sakata, *Professor*  
Masahiro Ikegami, *Associate Professor*  
Hiroyuki Takahashi, *Associate Professor*  
Yukiko Kanetsuna, *Assistant Professor*  
Lu Tomoe, *Assistant Professor*

Masaharu Fukunaga, *Professor*  
Masafumi Suzuki, *Associate Professor*  
Satoru Chiba, *Associate Professor*  
Koichi Nomura, *Assistant Professor*  
Tohru Harada, *Assistant Professor*

### General Summary

The research projects of our department focus on the pathogenesis, histogenesis, morphogenesis, and clinical pathology of nonneoplastic and neoplastic human diseases by means of light and electron microscopy, morphometry, immunohistochemistry, and gene analysis.

### Research Activities

#### *Pathology of the liver*

We continued to study the evolution of fibrosis in nonalcoholic steatohepatitis (NASH). Previous annual reports have found that bridging fibrosis develops between adjacent central veins and that portal tracts tend to be well preserved in the early stage. Because NASH leads to liver cirrhosis, this early stage could be called the non-cirrhotic stage. Furthermore, we used the tissue reconstruction method to examine at autopsy a case of NASH showing conspicuous fibrosis. We found that most bridging fibrosis was central-to-central bridging fibrosis, whereas portal-to-portal bridging fibrosis was rare. As a result, peripheral portal tracts were in the fibrous mesh formed by central-to-central bridging fibrosis. The pattern reminds us of so-called congestive cirrhosis. On the other hand, we found that small arteries developed around the central and hepatic veins. These arteries are apparently derived from preexisting arteries in the portal tracts. The histologic features provide us with useful data for considering the self-assembly of liver tissue in the thermodynamic nonequilibrium system.

We also studied the ballooning degeneration of hepatocytes to clarify the pathogenesis and progression of NASH. Ballooning cells were conspicuous in the centrilobular regions and fibrotic lesions. However, they were neither essential for NASH nor related to the fatty changes of hepatocytes and inflammation.

The relation of the development of hepatocellular carcinoma to the abnormal vasculature of the liver was examined at autopsy in 2 cases. However, a clear conclusion could not be obtained, and further studies are needed.

#### *Renal pathology*

The site and pattern of tissue damage were examined in idiopathic and drug-induced interstitial nephritis. Analysis showed that the damage tended to appear in the medullary ray region.

Histologic evaluation of specimens of renal cell carcinoma collected in the department was continued with revised general rules for clinical and pathological studies of renal cell carcinoma. In particular, the stage was reevaluated according to a newly revised rule.

#### *Gastrointestinal pathology*

A total of 5,058 colon polyps accumulated in the past 5 years were examined pathologically. Of these polyps, 4,363 (86.2%) were neoplastic and 696 (13.8%) were nonneoplastic. The most numerous nonneoplastic polyps were hyperplastic polyps, followed by juvenile polyps.

#### *Lung pathology*

We continued to investigate morphologic changes of lungs with centrilobular emphysema by means of thick histologic sections stained with Elastica-van Gieson. Histologic reconstruction was performed with both thin and thick sections to observe angioarchitectural changes in pulmonary emphysema. In normal lung, the pulmonary arteries gave rise to branches and were regularly distributed. Pulmonary arteries and veins were interdigitated with each other. On the other hand, in advanced lesions in pulmonary emphysema, the density of small pulmonary arteries clearly decreased. In addition, suspended arteries that had lost alveolar attachment appeared in cystic centrilobular lesions. These changes were thought to reflect the destruction of the pulmonary structure beginning from alveolar damage.

#### *Urogenital pathology*

The relation of expression of phosphorylated AKT (pAKT) and Ets-related gene (ERG) by cancer cells was investigated in prostate cancer. The expression of pAKT was recognized in 50% of prostate carcinomas from Japanese patients, whereas the expression rate of ERG was 25% in the same specimens. The staining intensities of pAKT and ERG showed a reciprocal relation. This finding suggests that these 2 carcinogenesis pathways are unrelated and independent.

A comparison of the incidence, volume, and patient age was made between cases of prostate cancer in specimens obtained at autopsy in the 1980s and from 2008 through 2011. We found that the incidence and the volume were greater in 2008 through 2011 than in the 1980s.

The TMPRSS2-ERG fusion gene is reportedly involved in the development of prostate cancer in American and European patients. The presence or absence of the fusion gene was examined in Japanese patients with prostate cancer.

#### *Gynecological pathology*

Clinicopathologic examinations were performed of 50 atypical polypoid adenomyomas (APAMs). The APAMs showed various histologic features. For example, in 1 case the tumor progressed into the myometrium, and in another case of APAM that had developed in an adenomyoma. In 15 cases APAMs were associated with adenocarcinomas that had a good prognosis. Curettage specimens often made diagnosis difficult. Sometimes,

APAM was misdiagnosed as endometrioid adenocarcinoma. Hysterectomy is the first choice for treatment.

### *Breast pathology*

A total of 191 cases of breast cancer of borderline malignancy were collected and studied with immunohistochemical staining for actin, p63, and CD10. When cells positive for these antibodies are present inside an intraductal proliferative lesion, the lesion is thought to be a benign tumor, papilloma. However, differentiating benign and malignant lesions is difficult when the positive cells are present along the luminal margin of the duct and are not found inside an intraductal proliferative lesion.

### *Oncology*

Expression of the protein prominin 1 (PROM1) was examined in hepatocellular carcinoma. Expression of PROM1 is significantly down-regulated in primary lesions compared with the corresponding nonneoplastic regions. No difference was found in PROM1 protein expression between primary tumor lesions and metastatic lesions. These results suggest that PROM1 is involved in the carcinogenesis of hepatocellular carcinoma.

### *Others*

Clinicopathological studies were performed of a case of rectal goblet cell carcinoid associated with ganglion neuroma, a case of benign mesenchymal tumor of the stomach, and a case of primitive neuroectodermal tumor of the prostate.

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## Department of Virology

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Kazuhiro Kondo, *Professor*

### General Summary

Human herpesvirus (HHV) 6, which can establish lifelong latent infections of hosts, is frequently reactivated. We are studying the molecular mechanisms of the latency and pathogenesis of HHV-6. Additionally, we are using HHV-6 and HHV-7 to study the mechanism of fatigue and as viral vectors for gene therapy.

Fatigue is an indispensable biological “alarm” for avoiding the state of exhaustion that is caused by severe stress and overwork and might also induce a variety of diseases. We have investigated the molecular mechanisms of the reactivation of HHV-6 and HHV-7, which are stimulated by fatigue, and identified the molecule that can induce viral reactivation during fatigue.

Using our understanding of HHV reactivation, we have developed a method for measuring the accumulation of fatigue by determining the amounts of HHV-6 and HHV-7, which are reactivated and released into the saliva.

### Research Activities

*Assessment of work-related long-term fatigue and differentiation from chronic fatigue syndrome by using salivary HHV-6 and HHV-7 reactivation as a biomarker*

Fatigue is composed of physical weakness, brought about by stress and other factors, and feelings of fatigue, such as exhaustion and tiredness. Long-term fatigue can be caused by work-related chronic stress, whereas chronic fatigue syndrome (CFS) can be triggered by an infection that results in feelings of fatigue that continue for a long period. These conditions can lead to a reduction in manpower and other social problems. To date, no effective and objective method has been developed to assess long-term fatigue. Moreover, long-term fatigue has been difficult to distinguish from CFS, which is also characterized by feelings of fatigue.

To develop an objective method of quantifying work-related long-term fatigue, we investigated the use of the HHV-6 and HHV-7, which are reactivated by fatigue or stress. The results showed an increase in salivary HHV-6 DNA copy numbers which correlated with the number of hours of office work. Research on Japanese Self-Defense Forces personnel whose workload was fully controlled showed that an increase in the amount of training produced a reversible increase in salivary HHV-6 and HHV-7 DNA copy numbers.

An investigation, in an animal model, of the molecular mechanism of HHV reactivation by fatigue showed an increase in inflammatory cytokines, a phenomenon already thought to play a part in the molecular mechanism of fatigue. The results also demonstrated a relationship between fatigue and the novel phenomenon of the induction of differentiation markers in myeloid cells, which are latent infection sites for this group of viruses.

Furthermore, in patients with CFS, we observed almost no increase in salivary HHV-6 and HHV-7 DNA copy numbers, demonstrating that fatigue in CFS and long-term work-related fatigue have different characteristics. These results suggest that salivary HHV-6 and HHV-7 reactivation, which is a simple and objective biomarker of long-term fatigue, might also help increase our understanding of the molecular mechanism of fatigue and improve the diagnosis of CFS.

#### *Novel gene therapy viral vector using nononcogenic lymphotropic herpesvirus*

Despite the use of retroviral vectors, efficiently introducing target genes into immunocytes, such as T cells, is difficult. In addition, retroviral vectors carry risks associated with the oncogenicity of the native virus and the potential for introducing malignancy in recipients due to genetic carryover from immortalized cells used during vector production. To address these issues, we have established a new virus vector that is based on HHV-6, a nononcogenic lymphotropic HHV that infects CD4+ T cells, macrophages, and dendritic cells. In the present study, we altered the cell specificity of the resulting recombinant HHV-6 by knocking out the U2-U8 genes. The resulting virus proliferated only in activated cord blood cells and not in peripheral blood cells. Umbilical cord blood cells produced replication-defective recombinant virus in a sufficiently high titer to omit the use of immortalized cells during vector production. The HHV-6 vectors led to high rates (>90%) of gene transduction in both CD4+ and CD8+ T cells. These viruses showed low-level replication of viral DNA that supported greater expression of the induced genes than that of other methods but that was insufficient to support the production of replication-competent virus. Furthermore, HHV-6 vectors containing short hairpin RNAs against CD4 and human immunodeficiency virus (HIV) Gag markedly inhibited the production of these proteins and of HIV particles. Our results demonstrate the utility of HHV-6 as a new noncarcinogenic viral vector for treating immunologic diseases and for immunotherapy.

#### *Identification of SITH-1 as novel latent protein of HHV-6 associated with CFS and mood disorders*

HHV-6 has exhibited the most promise as a candidate CFS-associated virus. We identified a novel HHV-6 latent transcript that was expressed during the relatively activated latent stage (intermediate stage) of HHV-6 latency. This transcript encoded the small open reading frame named small protein encoded by the intermediate transcript of HHV-6 (SITH) 1. In the present study we aimed to identify SITH-1 responsible for CFS. In addition, to determine the function of SITH-1 in the brain, we analyzed the behavior of mice that expressed SITH-1 in the brain.

We have studied the expression of SITH-1 by examining the prevalence of anti-SITH-1 antibodies in persons with CFS or mood disorders and in healthy persons. Antibody detection was by indirect immunofluorescence and enzyme-linked immunosorbent assay. 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 mice were analyzed with the tail suspension test, prepulse inhibi-

tion, and locomotor activity.

With an indirect fluorescent antibody method, the rate of SITH-1 positivity was high in patients with CFS or mood disorders. In addition, enzyme-linked immunosorbent assay showed a high correlation. In behavioral experiments, 3-week-old SITH-1 mice showed decreased immobility time in the tail suspension test and impaired prepulse inhibition. Meanwhile, 5-week-old SITH-1 mice showed a decrease in spontaneous motor activity and an increase in immobility time in the tail suspension test. Therefore, astrocytes exposed to SITH-1 seem to play a major role in depressive and manic-like behavior of mice. These results suggested that SITH-1 is involved in the onset of mood disorders.

## Department of Bacteriology

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Yoshimitsu Mizunoe, *Professor*  
Tadayuki Iwase, *Assistant Professor*

Akiko Tajima, *Assistant Professor*

### General Summary

Research projects of our department have focused on: 1) the analysis of *Staphylococcus aureus* biofilm formation, 2) the analysis of biofilm detachment factor secreted by *S. aureus*, 3) the molecular mechanisms of *S. aureus* biofilm disassembly triggered by *Staphylococcus epidermidis* Esp, 4) the effects of bacteriocins against methicillin-resistant *S. aureus* (MRSA) biofilm, 5) the mechanism of *Escherichia coli* O157 entering a viable but nonculturable (VNC) state, and 6) the mechanism of bacterial ATP secretion.

### Research Activities

#### *Intranasal application of S.epidermidis prevents MRSA in mice*

Recently, MRSA has emerged as a leading cause of infection worldwide. Colonization with MRSA predisposes to infection and facilitates transmission of the pathogen; however, available treatments do not prevent MRSA colonization. Studies of human nasal flora suggest that resident bacteria play a critical role in limiting *S. aureus* colonization and prompted us to ask whether application of commensal resident bacteria can prevent nasal colonization by MRSA. We established a murine model to answer this question and showed that mice with nasal precolonization by *S. epidermidis* became more resistant to colonization by MRSA. Our study suggests that application of commensal bacteria and antibiotics is a more effective strategy for preventing MRSA colonization.

#### *Analysis of biofilm detachment factor*

The bacteria within a biofilm matrix are protected from the host immune system and from antibiotic attack. Therefore, a substance that disassembles biofilms might have wide medical and industrial applications for preventing or eradicating biofilms. We found that *S. aureus* secretes a factor that causes its own biofilm to detach. The culture supernatant of *S. aureus* also detached the biofilms of *S. epidermidis*, MRSA, *Pseudomonas aeruginosa*, and *E. coli*. The factor responsible for the detachment effect has a molecular weight less than 500 Da and is heat-stable. The culture supernatant was fractionated with gel filtration chromatography and subjected to reverse-phase column chromatography. The flow-through was subjected to hydrophilic interaction chromatography and eluted with decreasing concentrations of acetonitrile (90% to 0%). The fraction that had detachment activity was analyzed with mass spectrometry. We are now attempting to identify the factor.

*Molecular mechanisms of S. aureus biofilm disassembly triggered by S. epidermidis Esp*  
*S. aureus* is frequently found in the nasal cavities of healthy persons, but the colonization

often causes pathogenic infection. *S. aureus* exhibits a strong capacity to attach to biotic or abiotic surfaces and to form biofilms, which lead to chronic infections. We have recently shown that Esp, a serine protease secreted by commensal *S. epidermidis*, inhibits *S. aureus* biofilm formation and nasal colonization. However, the substrate specificity and target proteins of Esp remain unclear. Therefore, the aim of this study was to elucidate these factors and thereby determine the mechanism by which Esp inhibits the formation of *S. aureus* biofilms. We used a mutant Esp protein (Esp<sup>S235A</sup>) with defective proteolytic activity; this protein did not disassemble the biofilm formed by a clinically isolated MRSA strain, thereby indicating that the proteolytic activity of Esp is essential for biofilm disassembly. Proteomic and immunological analyses showed that Esp degrades at least 73 proteins, including 11 proteins, such as extracellular adherence protein, fibronectin-binding protein A, protein A, and a putative lytic transglycosylase, associated with biofilm formation and colonization. Esp selectively degraded several human *S. aureus* receptor proteins (e.g., fibronectin, fibrinogen, and vitronectin) that are involved in its colonization or infection. These results suggest that Esp inhibits *S. aureus* colonization and biofilm formation by degrading specific proteins that are crucial for biofilm construction and host-pathogen interaction.

#### *Quality control of bacterial biofilm by extracellular molecular chaperones*

Proteomic analysis has been used to identify dozens of cytoplasmic proteins, including molecular chaperones DnaK and ClpB in the biofilm matrix fraction of MRSA. However, the biological significance of these chaperones in the extracellular environment is largely unknown. Here, we show the importance of these excreted chaperones in the quality control of biofilms. Both DnaK and ClpB were detected with Western blotting in the biofilm matrix fractions of various clinically isolated and laboratory *S. aureus* strains (n=49), and the abundance of these proteins varied among the strains. Indirect immunofluorescence microscopy revealed that DnaK and ClpB are attached to the cell surface of *S. aureus* embedded in biofilms. Interestingly, supplementation of media with DnaK and ClpB stimulated *S. aureus* biofilm formation in a dose-dependent manner. A cell-based pull-down assay demonstrated that the added DnaK and ClpB proteins were associated with *S. aureus* cells. In addition, DnaK and ClpB supported the development of biofilms produced by the clinically important pathogens *E. coli* and *P. aeruginosa*. These findings provide a novel insight into the extracellular functions of molecular chaperones DnaK and ClpB and also suggest the importance of these chaperones in infectious diseases.

#### *Amyloidosis mediated by bacterial biofilm infection*

Some bacteria secrete extracellular amyloid fibrils, leading to the production of robust biofilms on biotic or abiotic surfaces. In this study, we investigated a novel hypothesis that bacterial biofilm infection triggers amyloidosis in host organisms using an *E. coli* and *Caenorhabditis elegans* amyloidosis model. We found that *E. coli* curli amyloid fibrils promote aggregation of amyloid beta peptides *in vitro* and that feeding of curli-producing *E. coli* accelerates paralysis of *C. elegans* expressing amyloid beta peptide. These findings support our hypothesis.

### *Effects of bacteriocins against MRSA biofilm*

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by Gram-positive bacteria. Nisin, a bacteriocin produced by the lactic acid bacterium *Lactococcus lactis*, is used as a food preservative in many countries because it is nontoxic for humans and highly stable against heat and acids. In this study, we investigated the antibacterial activities of several kinds of bacteriocin (nisin A, lacticin Q, and nukacin ISK-1) against clinical isolates of biofilm-forming MRSA. After purified bacteriocins were added to MRSA biofilms, the antibacterial effects of the bacteriocins were analyzed with a colony-counting method and the live/dead staining of bacterial cells in biofilms. Vancomycin, which showed bacteriocidal activity against planktonic cells of MRSA, did not show activity against MRSA biofilm. On the other hand, nisin A showed high bactericidal activity against both planktonic cells and biofilm cells. Lacticin Q also showed bacteriocidal activity against both planktonic cells and biofilm cells, but its activity against biofilm was 1/10th that of nisin A. Nukacin ISK-1, which showed bacteriostatic activity against planktonic cells of MRSA, did not show activity against MRSA. Nisin A and lacticin Q are known to kill bacterial cells by forming pores on cytoplasmic membranes of target bacteria. Our results suggest that pore-forming bacteriocins are highly potent for the treatment of MRSA biofilm infections.

### *High-throughput screening of antibiofilm compounds*

Potential strategies for preventing and treating *S. aureus* biofilm infections are to use small molecules to inhibit biofilm development or to promote biofilm dispersal without the use of lethal selection pressure. High-throughput screening (HTS) could be used to identify other compounds effective against *S. aureus* biofilm development. We will perform HTS in collaboration with the University of Tokyo, which has a chemically diverse small-molecule library (200,000 compounds). First, we have established a crystal violet staining assay of biofilm that is suitable for the HTS method. Additionally, we have designed a screening robot system that automates the dispensing of compounds to assay plates, cell culture handling, and activity measurement. Hereafter, HTS of antibiofilm compounds that are active against MRSA biofilm will be performed.

### *Mechanism of *E. coli* O157 entering a VNC state*

Some *E. coli* O157 strains become VNC under environmental stress conditions and escape detection by conventional culture methods. We showed that the addition of catalase to the culture media resuscitated O157 from a VNC state to a culturable state and that the decrease of sigma factor S activity (encoded by *rpoS* gene) caused bacteria to enter a VNC state. An *rpoS* gene knockout strain was generated from the O157 Sakai strain. The *rpoS* mutant strain entered a VNC state, but the wild-type Sakai strain did not.

### *Characterization of ATP-secreting bacteria from mice and humans*

We have reported that ATP-secreting bacteria are present in the intestines of mice and humans. However, the mechanisms of ATP secretion in bacteria are not completely understood. We are investigating the mechanisms of bacterial ATP secretion.

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expression of immunity genes. *Appl Microbiol Biotechnol*. 2012; **93**: 671-8.

**Nishikori S, Esaki M, Yamanaka K, Sugimoto S, Ogura T.** Positive cooperativity of the p97 AAA ATPase is critical for essential functions. *J Biol Chem*. 2011; **286**: 15815-20.

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## Department of Public Health and Environmental Medicine

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Hiroyuki Yanagisawa, *Professor*  
Machi Suka, *Associate Professor*

Toshihiko Agata, *Associate Professor*  
Yuichi Miyakoshi, *Assistant Professor*

### General Summary

Our major research projects in the 2011 academic year focused on: 1) analysis of oxidative DNA damage, 2) evaluation of mutagenic potential related to diabetes mellitus (DM), 3) a method for analyzing 8-hydroxy-deoxyguanosine (8-OHdG) with matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI/TOF MS), 4) evaluation of fatigue following the compressed air work using human herpesvirus (HHV) 6 in saliva, 5) evidence-based medicine (EBM), 6) a questionnaire survey on drug information, 7) prevalence of menopausal symptoms, 8) associations between body weight and cardiovascular risk factors, 9) ecological studies of suicide, 10) annual changes in the suicide mortality rate in Japan, 11) effects of L-carnosine and its zinc complex polaprezinc on pressure-ulcer healing, 12) long-term follow-up study of type 2 DM, and 13) mental health in the workplace.

### Research Activities

#### *Experimental Medicine*

##### 1. Analysis of oxidative DNA damage

Analysis of 8-OHdG, an indicator of oxidative DNA damage, and deoxyguanosine (dG) was performed with a high-performance liquid chromatography-electrochemical detector/ultraviolet detector. The ratio of 8-OHdG/dG in zinc-excess rats was increased, compared with that in the control group. The ratio of 8-OHdG/dG in indium oxide ( $\text{In}_2\text{O}_3$ ) or indium hydroxide ( $\text{In}(\text{OH})_3$ ) was not increased.

##### 2. Evaluation of mutagenic potential related to DM

The effects of streptozotocin-induced DM in rats were studied with flow cytometry to measure micronuclei in both the reticulocyte and normochromatic erythrocyte populations in peripheral blood. The micronucleus frequency in DM rats tended to be higher even 4 weeks after treatment with streptozotocin. This result suggests that high blood glucose levels are associated with increased micronucleus frequency. In the future, more detailed studies will be required to confirm this finding, including its reproducibility for evaluating the risk of carcinogenesis by DM.

##### 3. A method for analyzing 8-OHdG with MALDI/TOF MS

The best indicator of the oxidation damage of DNA is 8-OHdG. Then, easurement of 8-OHdG with MALDI/TOF MS was done.

##### 4. Evaluation with HHV-6 in saliva of fatigue following work in compressed air

Many caisson workers report severe fatigue after working in compressed air. We used HHV-6 in saliva to evaluate the fatigue of these workers. Although working hours were short, HHV-6 DNA copy numbers after hyperbaric work were significantly higher than

those after normobaric work. The fatigue of workers in compressed air caissons might be induced by exposure to hyperbaric conditions.

*Epidemiology, EBM, investigation, and medical informatics*

1. EBM

A systematized body of epidemiologic principles with which studies can be designed and judged has been established only in the last 2 decades. These principles have evolved in tandem with an explosion of epidemiologic activity covering a wide range of health problems. Our greatest concern is to clarify risk factors for adult diseases and intractable diseases. We also studied the methodology of medical informatics education and EBM.

2. A questionnaire survey on drug information

A self-administered questionnaire was distributed to approximately 2,000 health examinees at a Japanese healthcare center to examine drug-information-seeking behavior of Japanese people and their attitudes toward drug information.

3. Prevalence of menopausal symptoms

Questionnaire surveys on menopausal symptoms were conducted among 50- and 60-year-old women who lived in northern Kawasaki. We determined the prevalence of menopausal symptoms among community-dwelling Japanese women and projected the number of women with menopausal symptoms in Japan from 2009 to 2055.

4. Associations between body weight and cardiovascular risk factors

Using 2008 and 2009 health examination data, we examined 1-year changes in body weight and cardiovascular risk factors among Japanese male workers. The effect of weight gain on cardiovascular risk factors was compared between younger (25–44 years) and older (45–64 years) groups.

5. Ecological studies of suicide

Using 2005 national census data, we examined the associations between age-adjusted suicide rates and socioeconomic factors in 47 prefectures and 358 medical care zones.

6. Annual changes in the suicide mortality rate in Japan

The suicide in Japan increased rapidly from 1998, and prevention of suicide is an important issue. Regional differences in suicide rates have previously been noted. The present study analyzed the relationship between 25 factors and the suicide mortality rate in each prefecture according to sex.

7. Effects of L-carnosine and its zinc complex polaprezinc on pressure-ulcer healing

We performed an interventional study examining the effects of L-carnosine and its zinc complex polaprezinc on the healing of chronic pressure ulcers. The results of this trial suggest that L-carnosine and polaprezinc are both beneficial for the treatment of pressure ulcers.

8. Long-term follow-up study of type 2 DM

We investigated the relationship between HbA1c variability and all-cause mortality in patients with type 2 DM. We recommend that attention should be paid to HbA1c variability as well as to the HbA1c level in the treatment of these patients, even if they are not elderly.

9. Mental health in the workplace

Mental health in the workplace is increasingly recognized as a serious problem. Several

questionnaires have been created to assess mental health in Japan. Concrete questions are important for managing stress in the workplace. The purpose of this study was to investigate stress in the workplace with a new questionnaire.

## Publications

**Ozaki S, Atsumi T, Hayashi T, Ishizu A, Kobayashi S, Kumagai S, Kurihara Y, Kurokawa MS, Makino H, Nagafuchi H, Nakabayashi K, Nishimoto N, Suka M, Tomino Y, Yamada H, Yamagata K, Yoshida M, Yumura W, Amano K, Arimura Y, Hatta K, Ito S, Kikuchi H, Muso E, Nakashima H, Ohsone Y, Suzuki Y, Hashimoto H, Koyama A, Matsuo S, Kato H.** Severity-based treatment for Japanese patients with MPO-ANCA-associated vasculitis: the JMAAV study. *Mod Rheumatol.* 2012; **22**: 394-404. Epub 2011 Sep 18.

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related quality of life in MPO-ANCA-associated vasculitis patients treated with cyclophosphamide plus prednisolone: an analysis of 18 months of follow-up data from the JMAAV study. *Mod Rheumatol.* 2012 Feb 4. Epub ahead of print.

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## Department of Forensic Medicine

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Kimiharu Iwadate, *Professor*

Kenji Fukui, *Assistant Professor*

### General Summary

Our main research projects in 2011 have focused on sudden unexpected infant death due to milk aspiration, diagnosis of drowning by detection of specific DNA fragments of aquatic bacteria from blood samples, analysis of the ubiquitin proteasome system and the autophagy lysosome system in the central nervous system, identification of war-dead remains through DNA analysis, the objective evaluation of the limits of DNA typing based on the intensity of ninhydrin treatment, and quantitative analyses of medicines and poisonous substances in forensic autopsy cases.

### Research Activities

#### *Forensic pathology*

##### 1. Sudden unexpected infant death due to milk aspiration

To examine longitudinal changes in the pathological findings of the lung and other organs in cases of milk aspiration, we performed an experimental study in a murine model. Immunostaining with an antibody against human  $\alpha$  lactalbumin showed reactions over time in the lung, kidney, and spleen. Detection of aspirated milk in organs other than the lung would be clear evidence of intravital milk aspiration and suggests previous or recurrent milk aspiration.

##### 2. Diagnosis of drowning by detection of specific DNA fragments of aquatic bacteria

Death by drowning is generally diagnosed on the basis of diatoms detected in organs other than the lung. We speculate that bacteria are more useful than plankton as markers for diagnosing death by drowning. From the preserved blood samples of 30 cases of freshwater drowning, specific DNA fragments of *Aeromonas sobria*, a common aquatic bacteria, were examined by means of the polymerase chain reaction. The DNA fragments of the bacterium were detected in most cases with the nested polymerase chain reaction.

##### 3. Analysis of the ubiquitin proteasome system and the autophagy lysosome system in the central nervous system

Research associated with the ubiquitin proteasome system and the autophagy lysosome system, which play major roles in the degradation of intracellular proteins and organelles, has advanced in the various areas of medical science. Autopsies cases of traumatic intracranial injury at the Department of Forensic Medicine were examined regarding how the ubiquitin proteasome system and the autophagy lysosome system are induced in traumatic intracranial injury. Both degradation pathways were induced in the injured cerebral cortex soon after trauma, and the pathway involved in the degradation of unnecessary substances or the cells in which degradation is activated were suggested to be different or altered over time after the traumatic event in the central nervous system.

### *DNA analysis*

#### 1. Identification of war-dead remains by means of 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. The objective evaluation of the limit of DNA typing based on the intensity of ninhydrin treatment

Shed epithelial cells on a sheet of paper were stained with ninhydrin reagent, and DNA typing was performed. We studied the relationship between the intensity of the purple staining after ninhydrin treatment and the limit of DNA typing as mitochondrial DNA polymorphisms, and we performed an objective evaluation to determine the target of the staining area for DNA analysis.

### *Forensic toxicology*

#### 1. Quantitative analyses of medicines and poisonous substances

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

#### 2. Examination of a method for analyzing sertraline

We detected sertraline in 4 cases at autopsy. Sertraline is an antidepressant of the selective serotonin reuptake inhibitor class. Qualitative and quantitative methods of analyzing sertraline with gas chromatography/mass spectrometry were examined. With quantitative analysis, high concentrations of sertraline were detected.

### *Radiocarbon analysis*

#### 1. Establishment of age estimation

We studied the estimation of date of birth from the quantity of radiocarbon isolated from tooth enamel. We have succeeded in specifying the age range from only a single tooth by measuring carbon-14 separately in incisal (occlusal) and cervical regions of the enamel.

### **Publications**

**Maebashi K, Iwadate K, Sakai K, Takatsu A, Fukui K, Aoyagi M, Ochiai E, Nagai T.** Toxicological analysis of 17 autopsy cases of hydrogen sulfide poisoning resulting from the inhalation of intentionally-generated hydrogen sulfide gas. *Forensic Sci Int.* 2011; **207**: 91-5.  
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findings of milk aspiration: an experimental study using a murine model. *Forensic Sci Int.* 2011; **209**: 183-5.

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## Department of Tropical Medicine

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Hiroataka Kanuka, *Professor*  
Masahiro Kumagai, *Assistant Professor*

Kenji Ishiwata, *Associate Professor*

### General Summary

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

### Research Activities

#### *Analysis of mucosal immunity to gastrointestinal nematode infection*

The intestinal tract possesses an immune system that regulates elimination and tolerance to various kinds of foreign substances, such as foods and microorganisms, coming from the mouth. The uniqueness of the intestinal tract's natural and acquired immunity, differing from systemic immunity, is being revealed. Interestingly, most types of human intestinal parasitosis are chronic infections that readily recur. The mucosal immune system has difficulty eliminating intestinal parasites as pathogens. In a mouse model, *Nippostrongylus brasiliensis* infection terminates within 2 weeks and leads to protective immunity against reinfection which is dependent on the Th2 immune response. Dendritic cells (DCs) and T cells in mesenteric lymph nodes were both activated 1 day after nematode establishment in the small intestine. The Th2 response, estimated by interleukin 4 production in the mesenteric lymph nodes, was detected at least 3 days after establishment, and the worms were expelled by 7 days after establishment. Interestingly, DCs decreased their MHC class II expression on cell surfaces after the transient activation. Antigen-presenting ability to specific T cells, followed by clonal proliferation, of DCs 5 days after establishment was half that of DCs 1 day after establishment. CD4-positive DCs, which are supposed to present foreign antigens to T cells, disappeared at the same time. These findings are under evaluation by comparing immune responses to *Heligmosomoides polygyrus* infection whose establishment continues more than one month in the mouse small intestine.

#### *Transcriptome analysis of Entamoeba using an ultrafast sequencer*

We have been performing transcriptome analysis of *Entamoeba histolytica* and *Entamoeba invadens*. *E. invadens* is a parasitic amoeba of reptiles which has been used as an alternative model to the encystation of *E. histolytica*, because *E. invadens* has a morphology and life cycle similar to those of *E. histolytica* and can be easily induced to

undergo encystation in an in vitro axenic liquid culture; in contrast, *E. histolytica* rarely forms cysts in vitro. At the outset, we tried to clarify whether the 5' untranslated region (5'UTR) of messenger RNA is as short as indicated by the analysis of a small number of genes. Through cooperative research, sequencing and bioinformatic analysis with various technologies were performed. We have been following sequences of the 2 species: 1) full-length complementary (c) DNA sequences without deletion of the 5' ends with the oligocapping method; 2) large quantities of tag sequences of 35 nucleotides starting from the transcription start site (TSS) obtained with TSS-Seq, a method that combines the advantages of oligocapping method and next-generation sequencers; and 3) RNA shotgun sequence assembly with RNA-Seq. These sequences were mapped on the genome sequences of *E. histolytica* and *E. invadens*. Previously, we published a database for full-length cDNAs of *E. histolytica* and *E. invadens* (<http://fullent.hgc.jp/>). Now we have integrated high-throughput data with our full-length cDNA database (<http://fullent.genome.ad.jp>). Integrated tools help users to visualize, search, and download the data. We then confirmed that the 5'UTRs of the genes of *E. histolytica* and *E. invadens* are uniformly short (about 10 nt on average). It became clear with TSS-seq that TSSs are not always fixed but can show variations. RNA-Seq demonstrated the alternative splicing between trophozoites and cysts in some genes of *E. invadens*. More than 500 cDNA sequences were mapped on intergenic regions of *E. histolytica* and *E. invadens*. Analyses of protein families (Pfam database) and RNA families (Rfam database) showed that 29 cDNA sequences were coding sequences of new genes that had not been predicted, 4 were of transfer RNA genes, and 1 was of a 5S ribosomal RNA gene. The other sequences are probably those of noncoding RNA, although structural analysis will be necessary for confirmation.

#### *Amino acid-related host nutrition dynamics during malaria infection*

Malaria parasites, which disable and kill more than 1 million people every year repeat division and multiplication in erythrocytes, taking in nutrition from an immediate environment. Free amino acids in the blood plasma might play an important role in the establishment of blood-borne parasites, because the biosynthetic pathways for most amino acids are absent in parasites, which rely on exogenous amino acids for most of their growth requirements. We demonstrated that plasma aminograms, which show the free amino-acid pattern of the blood plasma, change markedly as parasitemia and mortality increase, resulting in infection-dependent nutrition dynamics that belong to different clusters. Moreover, comparison of preinfection plasma aminograms between BALB/c, C57BL/6/J, and C3H/HeN mice, which have different sensitivities to malaria, showed that the concentrations of a subset of amino acids increase in proportion to malaria sensitivity. Because amino acid metabolic pathways are constructed as complicated nutritional networks, multivariate interference of amino acids might define the infectious disease process. Our results suggest that plasma aminograms strongly correlate with malaria parasite infection.

*Intraspecific diversity of midgut commensal bacteria in Anopheles mosquitoes defines Plasmodium transmission capacity*

A critical stage in malaria transmission occurs in the *Anopheles* mosquito midgut, when the malaria parasite, *Plasmodium*, ingested with blood, first makes contact with the gut epithelial surface. To develop novel strategies for controlling malaria, an understanding of the response mechanisms within the midgut environment, including those influenced by resident microbiota against *Plasmodium*, is needed. Here we focus on a midgut bacteria species' intraspecific variation that confers diversity to the mosquito's competency for malaria transmission. *Serratia marcescens* isolated from either laboratory-reared mosquitoes or wild populations in Burkina Faso shows great phenotypic variation in its cellular and structural features. Importantly, this variation is directly correlated with the ability of *S. marcescens* to inhibit *Plasmodium* development within the mosquito midgut. Furthermore, this anti-*Plasmodium* function conferred by *S. marcescens* requires increased expression of the flagellum biosynthetic pathway, which is modulated by the motility master regulatory operon, *flhDC*. These findings point to new strategies for controlling paratransgenic malaria through genetic manipulation of midgut bacteria within the mosquito.

*Odor-based contagious transmission of pathogen by fly*

The housefly and flies in general are considered to be mechanical vectors of many kinds of pathogens, whereas the mosquito serves as the biological vector for those pathogens. Mechanical vectors simply convey pathogens and are not essential for their development or life cycle. To clarify the molecular mechanisms of transmission by fly, we first established a model system for transmission using *Drosophila melanogaster*. Green fluorescent protein-labeled *Escherichia coli* located on the center of an agar-plate was freely ingested by *Drosophila*. Substances excreted in the feces are easily observed as small fluorescent spots on the surface of agar, showing that flies directly feed on *E. coli* and disseminate it by excretion. Flies without antennae, which contain a large set of olfactory receptors, and flies deficient for *Or83b*, which encodes a broadly expressed odorant receptor, showed impaired dissemination of bacteria. Whereas wild-type flies showed behavioral responses to attractive odors released from growing *E. coli*, the *Or83b*-deficient mutants failed to respond to these odors. Volatile compounds emitted from culture supernatant of *E. coli* were trapped and identified with gas chromatography-mass spectrometry. The predominant compound produced by *E. coli* was indole, along with lesser amounts of alcohols. We also showed that LUSH, the *Drosophila* orthologue of indole-binding protein, is required for transmission of *E. coli* as excreted droplets. Given that *Drosophila* LUSH is also known as a component to activate pheromone-sensitive neurons, we suggest that the pheromone-mediating system also promotes feeding behavior in the presence of indole from pathogens, contributing to the transmission of infectious diseases, such as food poisoning.

## Publications

**Kuranaga E, Matsunuma T, Kanuka H, Take-moto K, Koto A, Kimura K, Miura M.** Apoptosis controls the speed of looping morphogenesis in *Drosophila* male terminalia. *Development*. 2011; **138**: 1493-9.

**Doi Y, Shinzawa N, Fukumoto S, Okano H, Kanuka H.** Calcium signal regulates temperature-dependent transformation of sporozoites in malaria parasite development. *Exp Parasitol*. 2011; **128**: 176-80.

**Makioka A, Kumagai M, Hiranuka K, Kobayashi S, Takeuchi T.** Different structure and mRNA expression of *Entamoeba invadens* chitinases in the encystation and excystation. *Parasitol Res*. 2011; **109**: 417-23.

**Sawaguchi M, Tanaka S, Nakatani Y, Harada Y, Mukai K, Matsunaga Y, Ishiwata K, Oboki K, Kambayashi T, Watanabe N, Karasuyama H, Nakae S, Inoue H, Kubo M.** Role of mast cells and basophils in IgE responses and in allergic airway hyperresponsiveness. *J Immunol*. 2012; **188**: 1809-18.

## Reviews and Books

**Yokoyama T, Aonuma H, Kanuka H.** Immune system of vector mosquito (in Japanese). *Kagaku to Seibutsu*. 2012; **50**: 196-202.

**Kumagai M, Nishino T.** Malaria (in Japanese). *Medical Practice*. 2011; **28** Suppl: 506-9.

## Department of Laboratory Medicine

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Satoshi Kurihara, *Professor*  
 Akihiro Ohnishi, *Professor*  
 Ken Kaito, *Associate Professor*  
 Hiroshi Yoshida, *Associate Professor*  
 Tomokazu Matsuura, *Associate Professor*

Masato Suzuki, *Professor*  
 Sadayori Hoshina, *Associate Professor*  
 Hironari Sue, *Associate Professor*  
 Kenichi Sugimoto, *Associate Professor*  
 Midori Kouno, *Assistant Professor*

### General Summary

Research projects in our department in 2011 were concerned with clinical physiology, clinical microbiology, clinical chemistry, hematology, cardiology, clinical cell biology, and clinical psychiatry. Research achievements in each division are described below.

### Research Activities

#### *Clinical physiology*

In the OLETF rat, a model of obesity-related diabetes, we found that rats that exercised regularly at the age of 5 to 19 weeks had a significantly lower body weight than sedentary control rats at the later age of 20 to 45 weeks. In contrast, rats that received food-restriction treatment showed a rapid gain in body weight when the treatment was discontinued. Immediately after the completion of the experiment, at the age of 46 weeks, exercised rats showed significantly lower body weight and body fat mass and enhanced activities of energy metabolism-related enzymes and uncoupling protein 3 mRNA expression in skeletal muscle compared with those of the sedentary and food-restricted groups. These results strongly indicate that many individuals who receive diagnoses of obesity or metabolic syndrome during adolescence to middle age may have had insufficient physical activity during childhood.

#### *Clinical microbiology*

Phage open reading frame typing (POT), a new typing method for methicillin-resistant *Staphylococcus aureus*, was compared with pulsed-field gel electrophoresis (PFGE), which is the standard method. The results suggest that the POT method has a discrimination power equal to that of PFGE.

A polymerase chain reaction (PCR) method targeted to the internal spacer region (ITS) was developed to detect *Cunninghamella* spp.

Several clinically isolated, previously unidentified bacterial strains were identified through gene sequencing of PCR-amplified 16S ribosomal RNA.

The isolation rate of *S. aureus* carrying the Panton-Valentine leukocidine (PVL) gene from patients with skin infections at Daisan Hospital investigated with PCR *luk-S-PV* and *luk-F-PV* amplification was 7%.

#### *Clinical chemistry*

1. To evaluate the clinical usefulness of the serum level of procalcitonin, a precursor of

calcitonin, for determining whether bacterial infection is present in patients with systemic inflammatory response syndrome (SIRS) caused by bacterial sepsis, the level of procalcitonin was measured concomitantly with the white blood cell count and levels of tumor necrosis factor- $\alpha$ , interleukin 6, E-selectin, and C-reactive protein. The subjects were 5 patients with SIRS not complicated by Gram-negative bacterial infection. We analyzed the time to reach peak level ( $C_{\max}$ ) from the clinically diagnosed time-point of SIRS ( $T_{\max}$ ) for the above 6 inflammatory markers. The mean  $T_{\max}$  was shortest for tumor necrosis factor- $\alpha$  (18.0 hours) and was followed by those for procalcitonin (32.9 hours), interleukin 6 (36.0 hours), the white blood cell count (36.7 hours), and C-reactive protein (43.0 hours), and was longest for E-selectin (46.5 hours). These findings suggest that measuring levels of procalcitonin in patients with SIRS is useful for diagnosing bacterial infection and the severity of illness.

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 methods for assessing cardiovascular disease risk, including the application of our method of high-performance liquid chromatography, to determine levels of lipoprotein cholesterol.

Our studies obtained the following results. 1) The method of high-performance liquid chromatography we developed has excellent quantitative performance for lipoprotein cholesterol in samples with increased remnant lipoprotein (*Atherosclerosis*, in press); 2) The pleiotropic effects of hydroxymethyl glutaryl coenzyme A reductase (statin) on oxidized lipoproteins vary, and pitavastatin can markedly decrease malondialdehyde-modified low-density lipoprotein/apolipoprotein B, whereas atorvastatin can decrease oxidized high-density lipoprotein/apolipoprotein A1 (under submission).

### *Hematology*

Because intravascular large B-cell lymphoma (IVLBCL) is difficult to diagnose, we evaluated the clinical characteristics of IVLBCL. In many cases hemophagocytic histiocytosis showing hemophagocytic syndrome is the most prominent finding that leads to the diagnosis, whereas in some cases small collections of lymphoma cells in the bone marrow and skin are an important sign of IVLBCL. Because many types of IVLBCL exist, systemic evaluation, including bone marrow aspiration and skin biopsy, is extremely important.

### *Cardiology*

We studied 2 topics in 2011. One was T-wave abnormalities in electrocardiograms, and another was the recurrence of atrial fibrillation after pulmonary vein isolation. We published several papers regarding new methods to prevent the recurrence of atrial fibrillation after pulmonary vein isolation.

### *Clinical cell biology*

1. We investigated the convenience and sensitivity of the fasting  $^{13}\text{C}$ -glucose breath test (FGBT) for evaluating hepatic insulin resistance. Healthy, nonobese subjects and a disease group of patients with mild glucose intolerance were given 100 mg of  $^{13}\text{C}$ -glucose

after an overnight fast. A series of breath samples was collected until 360 minutes after ingestion, and the  $^{13}\text{CO}_2/^{12}\text{CO}_2$  ratio was measured with an infrared spectrometer and plotted as a kinetic curve of  $^{13}\text{C}$  excretion. The area under the curve until 360 minutes ( $\text{AUC}_{360}$ ) of the  $^{13}\text{C}$  excretion kinetic curve of the FGBT reflects the efficiency of energy production of the liver. The  $\text{AUC}_{360}$  of the healthy subjects was consistently higher than that of patients with glucose intolerance. Insulin resistance in males and females could be diagnosed with a cut-off value. In a similar manner, diabetes mellitus could be diagnosed with cut-off values. The FGBT is a novel glucose metabolic test that can be used to conveniently and safely evaluate the balance of glucose metabolism in the liver. This test has excellent sensitivity for diagnosing alterations in hepatic glucose metabolism. In particular, the FGBT was useful for evaluating liver insulin resistance in cases of fatty liver. (Supported by the Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Strategic Research Foundation at Private Universities, 2011-2015) (in collaboration with Meiji University, National Defence Medical College, and the Departments of Internal Medicine and Surgery, The Jikei University)

2. We have developed a quantitative and specific assay for plasma latency-associated protein of transforming growth factor (TGF)  $\beta$  (LAP) degradates (LAP-Ds), which are produced during proteolytic TGF- $\beta$  activation. LAP-Ds would be novel markers in blood and tissues reflecting fibrogenetic activity but not the amount of accumulated fibrosis, particularly in patients with chronic hepatitis C virus infection and autoimmune hepatitis, and can be used to estimate fibrogenesis, in which TGF- $\beta$  activation and activation of hepatic stellate cells are more frequent, and to assess the effect of treatment. The plasma LAP-D concentrations decreased significantly when patients with chronic hepatitis C virus infection were treated with interferon and ribavirin. A sustained virological response was obtained when levels of LAP-D continued to be undetectable low. (Supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation) (In collaboration with the Institute of Physical and Chemical Research)

3. Because a large quantity of energy, particularly ATP, is necessary for protein secretion in hepatocellular carcinoma (HCC), we examined the production of energy from glucose in the FLC-4 and FLC-7 HCC cell lines. Glucose transporter (GLUT) 2 and glucokinase (GK), were expressed by FLC-4. This result indicates that FLC-4 cells are hepatocyte-type energy-producing cells that use the tricarboxylic acid cycle. Therefore, we speculated that FLC-4 uses energy for metabolism, not cell proliferation, under stable culture conditions in a radial-flow bioreactor (RFB). In contrast, FLC-7 showed carbohydrate metabolism depending on GLUT1 and hexokinase II. The findings suggest that energy-producing efficiency was poor and that glucose is used for cancer cell growth rather than energy production (Warburg effect). Because brain-type GLUT3 has high glucose-uptake efficiency expressed in the RFB culture, much glucose is used for cell proliferation. Therefore, it is necessary to consider culture methods other than RFB for protein production by FLC-7. (Supported by the Human Science Foundation) (In collaboration with the National Institute for Infectious Diseases, Waseda University, and the Department of Biochemistry, The Jikei University)

### Clinical psychiatry

The ability of psychotropic drugs to reduce the seizure threshold is a cause of concern in clinical practice; therefore, we examined the safety and efficacy of psychotropic drugs in several forms of psychosis associated with epilepsy. We reported on 2 patients with epilepsy and reflex seizures caused by their psychological state. Furthermore, we examined the characteristics of epileptic falls and dental and orofacial injuries in mentally handicapped patients. A study was performed to prevent the recurrence of depression in patients with epilepsy.

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## Department of Internal Medicine

### Division of Gastroenterology and Hepatology

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### Research Activities

#### *Alimentary tract*

#### 1. Photoimmunotherapy: A molecular target-specific theranostic phototherapy

We have recently developed photoimmunotherapy (PIT), a type of molecular target-specific phototherapy that uses monoclonal antibodies conjugated with near-infrared phthalocyanine dye. PIT has shown target-specific effects in preclinical studies in in-vivo tumor models. In addition, we have developed a repeated PIT method to eradicate targeted tumors.

#### 2. Efficacy of enemas with a zinc-containing compound, polaprezinc, in patients with ulcerative colitis

Endoscopic and clinical examinations show that the addition of polaprezinc (N-(3-aminopropionyl)-L-histidinato zinc) enemas produce significant improvements in patients with moderate-to-severe ulcerative colitis. Significant improvements were detected with endoscopy in the rectum and sigmoid colon, which are the areas exposed to the polaprezinc enema. Polaprezinc enemas may become a useful add-on treatment to accelerate mucosal healing in ulcerative colitis.

#### 3. Visualization of colorectal serrated lesions with image-enhanced endoscopy and immunohistochemical staining

Sessile serrated adenoma/polyp (SSA/Ps) has recently been reported to have malignant potential. However, distinguishing SSA/Ps from hyperplastic polyps is difficult with conventional endoscopy. If hyperplastic polyps could be distinguished from SSA/Ps by means of image-enhanced endoscopy, unnecessary endoscopic mucosal resection could be avoided. The purpose of this study was to examine the pathological features of colorectal serrated lesions on image-enhanced endoscopy with immunohistochemical staining.

#### 4. Photodynamic diagnosis of colitis-associated cancer or dysplasia in patients with ulcerative colitis through visualization following sensitization with oral 5-aminolevulinic acid

Our data, including results in a mouse model, suggest that photodynamic diagnosis with fluorescence endoscopy, following sensitization with orally administered 5-aminolevulinic acid, is a promising method for detecting lesions of colitis-associated cancer or dys-

plasia during ulcerative colitis surveillance.

#### 5. Elemental diet as a bowel preparation regimen for colonoscopy in patients with inflammatory bowel disease

Bowel preparation with an elemental diet (Elental<sup>®</sup>, Ajinomoto Pharmaceutical Co., Ltd., Tokyo) significantly reduces the volume of polyethylene glycol used for gut lavage. This modified regimen is safe and effective and has favorable patient acceptance. Thus, this bowel preparation regimen for colonoscopy is suitable for patients with inflammatory bowel disease.

#### 6. Arachidonic acid in patients with Crohn's disease

In patients with Crohn's disease, the mean percentage weight of arachidonic acid in the erythrocyte membrane was significantly higher and that of linoleic acid was significantly lower than those in healthy subjects, suggesting the possibility that delta 6-desaturase is hyperactivated in patients with Crohn's disease.

#### 7. The anatomical esophagogastric junction on endoscopic images

A human cadaver study showed that both mucosal ring B and gastric sling fibers constitute the fold around the cardiac orifice and that this fold could be defined as the anatomical esophagogastric junction on endoscopic images.

#### 8. Risk factors for lymph node metastasis in superficial esophageal carcinoma

Statistical analysis showed that the strongest risk factor for metastasis of esophageal superficial carcinoma to lymph nodes was vascular invasion, as indicated by a special staining procedure.

### *Liver*

#### 1. Clinicopathological study of primary biliary cirrhosis

We studied the clinicopathological characteristics of primary biliary cirrhosis. We statistically analyzed several blood chemical variables and histological findings found at liver biopsy.

#### 2. Nutritional imbalance in patients with liver cirrhosis

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

#### 3. Nonalcoholic fatty liver disease

The pathogenesis of nonalcoholic fatty liver disease resembles that of metabolic syndrome. We evaluated in detail the nutritional conditions in patients with nonalcoholic fatty liver disease and metabolic syndrome.

We have examined the new nutritional accurate method. The level of serum cytokeratin 18 fragments was significantly higher in nonalcoholic steatohepatitis than in simple steatosis. We examined the epidemiology of non-B non-C liver cirrhosis, which has continued to increase in prevalence. Heavy alcohol consumption in men and nonalcoholic steatohepatitis in women were identified as the main causes of non-B non-C liver cirrhosis. In alcoholic liver cirrhosis, high cumulative alcoholic consumption was related to the development of hepatocellular carcinoma (HCC).

#### 4. Sleep apnea syndrome and metabolic imbalance

Sleep apnea syndrome appears to be related to an imbalance of hepatic metabolism. Cases of sleep apnea syndrome were complicated by severe hypoxia and liver dysfunction. We analyzed the respiratory quotient with indirect calorimetry.

#### 5. Intrahepatic kinetics of natural killer T cells in a mouse model of autoimmune hepatitis

Natural killer T-cell activity is one of main pathogenesis in autoimmune hepatitis. Natural killers T cells and profiles of several cytokines were examined in a mouse model of AIH. Changes in immunoreactions were analyzed in knock-out mice.

#### 6. The relation between histological findings and biochemical laboratory data in viral hepatitis and autoimmune liver disease

In several cases of viral hepatitis and autoimmune liver disease histological activity and staging disagreed with biochemical data. We are considering the difference between detail histological findings and biochemical data.

#### 7. Treatment response of antiviral analogue nucleic acids in chronic hepatitis B virus infection

Resistant viral mutations are a serious problem in chronic hepatitis B virus infection treated with nucleic acid analogues. The gene sequences of the virus were analyzed. Furthermore, the response rate of the virus was studied. We considered the possibility of a new concurrent therapy for chronic hepatitis B virus infection.

#### 8. Pegylated interferon plus ribavirin therapy for chronic infection with hepatitis C virus G1b

Multiple regression analysis revealed that interleukin 28B polymorphism, the serum level of apolipoprotein B, and an amino acid substitution at position 70 of the hepatitis C virus (HCV) core region were independent factors associated with the efficacy of pegylated interferon plus ribavirin therapy on chronic infection with HCV G1b.

#### 9. HCC

Magnetic resonance was used to assess HCC. The magnetic resonance imaging properties with a contrast-enhancing medium were related to clinical and histological characteristics. The serum level of C-reactive protein before treatment was independently associated with overall survival. We found that the pretreatment serum C-reactive protein level is associated with tumor progression and reduced liver function and is an independent marker of poor prognosis in patients with HCC.

#### 10. The transforming growth factor $\beta$ activation reaction of the toxin receptor-mediated cell knockout model of acute hepatic failure

The transforming growth factor (TGF)  $\beta$  activation reaction is clearly detected in liver tissues of the toxin receptor-mediated cell knockout mouse model of acute hepatic failure, and the plasma level of latency-associated protein of TGF- $\beta$  degradates (LAP-Ds) reflects TGF- $\beta$  activation in liver tissues.

#### 11. The fasting $^{13}\text{C}$ -glucose breath test

Healthy, nonobese subjects and patients with mild glucose intolerance were given 100 mg of  $^{13}\text{C}$ -glucose after an overnight fast. A series of breath samples was collected until 360 minutes after ingestion, and the  $^{13}\text{CO}_2/^{12}\text{CO}_2$  ratio was measured with an infrared spectrometer and plotted as a kinetic curve of  $^{13}\text{C}$  excretion. The insulin resistance in males

and females could be diagnosed with a cut-off value. In a similar manner, diabetes mellitus could be diagnosed with a cut-off value. The fasting  $^{13}\text{C}$ -glucose breath test is a novel glucose metabolic test that can be used to conveniently and safely evaluate the balance of glucose metabolism in the liver. This test has excellent sensitivity for diagnosing alterations in hepatic glucose metabolism. In particular, the test was useful for evaluating liver insulin resistance in cases of fatty liver.

## 12. Quantitative and specific assay of plasma LAP-Ds

The LAP-Ds are novel markers in blood and tissues reflecting fibrogenetic activity but not the amount of accumulated fibrosis in patients, especially those with chronic HCV infection or AIH, and can be used to detect an early stage of fibrosis, when TGF- $\beta$  activation and hepatic stellate cell activation are more frequent, and to assess the effects of treatment. In particular, plasma LAP-D concentrations significantly decreased when patients with chronic HCV infection were treated with interferon-ribavirin combination therapy. A sustained virological response was obtained in cases in which the LAP-D concentration continued to be low and undetectable.

## Pancreas

Immunotherapy targeting Wilm's tumor protein 1 can mediate a potent antitumor effect when combined with chemotherapy for pancreatic cancer.

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## Department of Internal Medicine

### Division of Neurology

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#### General Summary

Our research in 2011 was conducted in the following areas: 1) abnormalities of movement and posture in patients with Parkinson's disease (PD), 2) autonomic dysfunction in neurodegenerative diseases, 3) neurophysiological studies of diabetic polyneuropathy and visual information processing functions in neurodegenerative diseases, 4) neuroradiological studies with nuclear medicine, 5) ultrasonographic studies of cerebrovascular disease, 6) cerebral infarctions in clear cell carcinoma of the ovary, and 7) basic research on motor neuron disease and axonal plasticity of the central nervous system.

#### Research Activities

##### *Abnormalities of movement and posture in patients with PD*

Zonisamide is an antiepileptic agent that has been used, on the basis of the results of clinical and experimental studies, to manage tremor in patients with PD in Japan since 2009. In the present study, we quantitatively evaluated the effectiveness of zonisamide for parkinsonian tremor with an actigraph, an instrument that can sense motion and record motor counts. Actigraphy was performed before and after treatment with zonisamide in patients with PD. The motor count after treatment with zonisamide was significantly lower with that before treatment, objectively showing that zonisamide is effective for treating tremor in PD. We conclude that zonisamide is promptly effective for controlling tremor in patients with PD.

We also surveyed antecollis, camptocormia, and other postural deformities in PD and related disorders as part of a multicenter investigation.

##### *Autonomic dysfunction in neurodegenerative diseases*

We studied cardiovascular autonomic dysfunction in patients with Lewy body diseases, such as PD and dementia with Lewy bodies. Autonomic nervous function, including cardiac sympathetic gain, was evaluated on the basis of cardiac uptake of radioiodinated metaiodobenzylguanidine, the response to the Valsalva maneuver, and the orthostatic tolerance test. Using these methods, we examined the characteristics of subclinical autonomic nervous dysfunction in de novo PD. We also studied the relation of olfactory dysfunction to cardiovascular dysautonomia in patients with PD. Olfactory dysfunction in PD was thus found to be significantly related to both cardiac sympathetic and parasympathetic dysfunction, as well as to vascular sympathetic dysfunction. As nonmotor

symptoms of PD, olfactory dysfunction and autonomic network failure appear to be closely related.

Our present study demonstrated marked impairment of olfactory sensation in Japanese patients with PD, as assessed with the simple, inexpensive, and noninvasive Odor Stick Identification Test for the Japanese. This test could be clinically useful for detecting olfactory dysfunction in PD and for differentiating PD from multiple system atrophy and progressive supranuclear palsy.

We investigated the volume of the olfactory bulb in PD and PD-related diseases, including multiple system atrophy, progressive supranuclear palsy, corticobasal degeneration, and other neurodegenerative diseases. The olfactory bulb volume in PD was smaller than that in other PD-related diseases. These results are compatible with those of our study of the olfactory bulb in autopsy cases. Magnetic resonance may be a useful tool for the differential diagnosis of PD and PD-related diseases. We plan to carry out further examinations.

We used the Parkinson Fatigue Scale (PFS-16) to compare fatigue and various clinical features of PD subtypes. We divided patients with PD into 2 groups: those with tremor-dominant PD and those with akinetic rigid PD. Using PFS-16, we compared the patient groups in terms of age, disease duration, Unified Parkinson's Disease Rating Scale, postural changes in systolic blood pressure, cardiac metaiodobenzylguanidine uptake, and coefficient variation of RR intervals. We further divided patients with tremor-dominant PD and those with akinetic rigid PD into subgroups—the F+ subgroup ( $\text{PFS-16} \geq 3.3$ ), and the F− subgroup ( $\text{PFS-16} < 3.3$ )—and investigated the differences between the 4 subtypes. Patients with akinetic rigid PD and fatigue had severe motor impairment and orthostatic hypotension. Therefore, akinetic rigid PD differs from tremor-dominant PD in terms of motor impairment, fatigability, and autonomic failure.

#### *Neurophysiological studies of visual information processing functions in neurodegenerative diseases and of diabetic polyneuropathy*

Visual information processing functions were compared in patients with PD, dementia with Lewy bodies, and Alzheimer's disease, by means of visual and auditory event-related potentials. The findings of the study suggest that in patients with PD and in patients with dementia with Lewy bodies with visual hallucinations, but not in patients with Alzheimer's disease, visual information processing functions are selectively impaired, compared with auditory functions.

Nerve conduction studies and neurological examination of the feet with newly established techniques in patients with diabetes mellitus who had no sensory symptoms in the feet were performed in collaboration with the Department of Diabetes, Metabolism and Endocrinology. The findings of the study suggest that 34% of these patients have subclinical polyneuropathy.

#### *Neuroradiological studies with nuclear medicine*

Neuroradiological studies were performed in patients with neurodegenerative disorders, including dementia and parkinsonism. Brain perfusion images were compared by means of statistical imaging methods, such as 3-dimensional stereotactic surface projection

analysis of isopropylidoamphetamine single-photon emission computed tomography (SPECT) images and the easy Z-score imaging system of ethyl cysteinate dimer SPECT images, among patients with dementia and parkinsonian disorders. These novel methods demonstrated the spectrum of pathological involvement of cholinergic and dopaminergic projections of Alzheimer's disease and PD, suggesting their usefulness for routine clinical practice.

#### *Ultrasonographic studies of cerebrovascular disease*

Cerebrovascular ultrasonography was useful for evaluating cerebral hemodynamics rapidly and in real time for patients with acute ischemic stroke. We evaluated the occlusion of intracranial and extracranial arteries with ultrasonography and monitored residual flow in real time every 15 minutes until 120 minutes after bolus administration of tissue plasminogen activator (t-PA). Two patients treated with t-PA had insufficient echo windows. In the first patient internal carotid artery occlusion was diagnosed with carotid ultrasonography. Because the occlusion persisted after t-PA therapy, endovascular therapy was considered. The second patient had ischemic stroke caused by cholesterol crystal embolism, which was indicated by a microembolic signal at the posterior cerebral artery. Monitoring of the microembolic signal with ultrasonography was useful for evaluating the therapeutic effect in this case of cholesterol crystal embolism. Real-time ultrasonographic monitoring is useful for evaluating the early thrombolytic effect of t-PA associated with early clinical recovery.

#### *Cerebral infarctions in clear cell carcinoma of the ovary*

Clear cell carcinoma of the ovary is a well-known cause of thrombosis and embolism. Clear cell carcinoma is reportedly complicated by pulmonary embolism more often than are other ovarian cancers. In this study we examined the complication of cerebral infarction in cases of ovarian cancer. We found that clear cell carcinoma was complicated by cerebral infarction more often than were other ovarian cancers.

#### *The mechanism underlying the selective vulnerability of motoneurons*

Amyotrophic lateral sclerosis is a fatal neurodegenerative disease characterized by progressive loss of motoneurons. Data suggest that selective vulnerability of motoneurons is due to an imbalance between excitatory and inhibitory innervation. Therefore, realizing the function and the development of inhibitory inputs on motoneurons is important for understanding selective vulnerability. We have performed experiments to study the plasticity of glycinergic synaptic inputs. We recorded the glycine synaptic membrane current from control mice and glycine receptor  $\alpha 3$ -deficient mice. Our findings suggest that glycine inputs increase with age and that glycine receptors ( $\alpha 3$ ) induce presynaptic plasticity.

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## Department of Internal Medicine

### Division of Kidney and Hypertension

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Tatsuo Hosoya, *Professor and Chairperson*  
Tetsuya Kawamura, *Associate Professor*  
Keitaro Yokoyama, *Associate Professor*  
Kazushige Hanaoka, *Assistant Professor*  
Yoichi Miyazaki, *Assistant Professor*  
Takashi Yokoo, *Assistant Professor*

Iwao Ohno, *Professor*  
Yasunori Utsunomiya, *Associate Professor*  
Makoto Ogura, *Assistant Professor*  
Masato Ikeda, *Assistant Professor*  
Hiroshi Hayakawa, *Assistant Professor*

### General Summary

Major fields of research are nephrology, hypertension, and uric acid metabolism. Published achievements and recent reports are summarized here.

### Research Activities

#### *Nephrology*

##### 1. Glomerulonephritis

We have established 4 histological grades corresponding to the percentage of glomeruli exhibiting pathological variables. Our evidence-based histological classification can identify the magnitude of the risk of progression to end-stage kidney disease and is useful for predicting long-term renal outcomes in immunoglobulin A (IgA) neuropathy.

We demonstrated that glomerular density in renal biopsies may be an important determinant of glomerular size variability and can influence the clinical phenotype in adult patients with minimal change nephrotic syndrome.

Dysregulation of vascular endothelial growth factor (VEGF) expression plays an important role in the pathogenesis of certain renal diseases. We generated and analyzed inducible and podocyte-specific VEGF transgenic mice using the Tet-On system. Our results demonstrate that the dysregulation of VEGF expression can alter the characteristics of both endothelial and mesangial cells, thereby leading to the impairment of glomerular capillary formation.

We determined the metabolic function of transplanted metanephroi with particular reference to maintaining blood pressure. We found that transplantation of metanephroi produces plasma rennin activity and contributes to raising blood pressure in a rat model of acute hypotension.

##### 2. Dialysis and kidney transplantation

We investigated the effects of cinacalcet on serum levels of Ca and P in patients undergoing hemodialysis with or without high levels of parathyroid hormone to control serum levels of Ca and P. We concluded that administration of cinacalcet to patients with or without high parathyroid hormone levels facilitates the control of Ca and P levels.

Encapsulating peritoneal sclerosis is a severe complication of long-term peritoneal dialysis (PD) and has a high mortality rate. We used a laparoscopic approach to evaluate peritoneal injury in patients undergoing PD. We found that PD peritonitis is a risk factor

for encapsulating peritoneal sclerosis and hypothesized that the bacterial species causing PD peritonitis changes depending on the neutral-pH PD solution.

We showed an association between peritubular capillary endothelial c-Jun activation and interstitial fibrosis in chronic antibody-mediated rejection.

We investigated the mechanism by which the intracellular  $\text{Ca}^{2+}$  concentration changes by applying drugs or by changing the extracellular  $\text{Ca}^{2+}$  concentration. Calcium oscillations may be associated with the function of renal tubular epithelial cells.

### *Hypertension*

The Jikei Optimal Antihypertensive Treatment (JOINT) study is a large-scale prospective interventional observational study that examined the effects of a fixed-dose combination of losartan and hydrochlorothiazide in patients with chronic kidney disease. The study has been finished and recruited a total of 280 participants. The main results have been published in *Clinical and Experimental Nephrology*. Additionally, as a subanalysis of the JOINT study, an extensional observation was performed on the relationship between uric acid and its associates.

A study of the effect of intensive antihypertensive therapy on the increased intrarenal-renin angiotensin system in patients with chronic kidney disease was designed and performed. The main finding was that both urinary protein and angiotensinogen were decreased in response to the intensive treatment.

Change in blood pressure during hemodialysis is not associated with water removal but is correlated with changes in hormones of the renin-angiotensin-aldosterone system (RAAS). Moreover, the response of RAAS hormones to fluid removal is improved in the presence of RAAS inhibitors, suggesting that RAAS blocking modulates the blood pressure-controlling mechanism in patients undergoing hemodialysis. This abnormal blood pressure regulation might be accelerated in the presence of diabetes.

### *Uric acid metabolism*

The urinary excretion of uric acid and sodium was examined in patients with IgA nephropathy. There were two types of patients: in one type urinary excretion of uric acid was significantly correlated with that of sodium (sodium-dependent type), and in the other type the urinary excretion of uric acid was not correlated with that of sodium (sodium-independent type). Renal tubule-interstitial damage was present only in patients with sodium-dependent-type IgA nephropathy.

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## Department of Internal Medicine

### Division of Rheumatology

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Akio Yamada, *Professor*  
Isamu Kingetsu, *Assistant Professor*

Daitaro Kurosaka, *Associate Professor*

#### General Summary

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

#### Research Activities

Clinical and experimental studies of autoimmune disease were performed.

1. Analysis of the relationship of neovascularization in animal models of autoimmune disease

Several studies have reported the arthritis-inhibiting effects of neovascularization inhibitors administered in animal models of rheumatoid arthritis. We evaluated the effects of the neovascularization inhibitor endostatin in murine models of collagen-induced arthritis and bleomycin-induced pulmonary fibrosis.

Furthermore, we are investigating whether *Bombina variegata* peptide 8 is involved in angiogenesis in autoimmune arthritis.

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

To assess synovial neovascularization in patients with rheumatoid arthritis, we evaluated the synovial blood flow signals on power Doppler ultrasonography and analyzed the correlation of neovascularization-related factors (e.g., vascular endothelial growth factor) with serum or disease activity.

3. Histopathological comparison between dermatomyositis and polymyositis

We obtained muscle, fascia, and skin via en-bloc biopsy from patients with dermatomyositis or polymyositis under magnetic resonance-guidance and then histopathologically investigated the severity of inflammation in muscle, fascia, and subcutaneous tissue to find differences between dermatomyositis and polymyositis.

4. Clinical studies aimed at standardizing immunosuppressant therapy of autoimmune disease

Many immunosuppressant drugs have been used to treat severe autoimmune diseases, such as amyopathic dermatomyositis with interstitial pneumonia, but the efficiency and treatment strategies of these drugs have not been clarified. We performed a clinical trial to establish a strategy for treating severe autoimmune diseases. Clinical studies aimed at standardizing the immunosuppressant therapy of autoimmune diseases were performed.

## Department of Internal Medicine

### Division of Cardiology

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 Teiichi Yamane, *Associate Professor*  
 Makoto Kawai, *Associate Professor*  
 Kimiaki Komukai, *Assistant Professor*  
 Taro Date, *Assistant Professor*

Mitsuyuki Shimizu, *Professor*  
 Shingo Seki, *Associate Professor*  
 Kenichi Hongo, *Associate Professor*  
 Takahiro Shibata, *Assistant Professor*  
 Takayuki Ogawa, *Assistant Professor*

### General Summary and Research Activities

Research in every field, both clinical and basic, is being driven daily on the basis of reliable results.

#### *Clinical research*

In clinical research, we have been participating in multicenter collaborative studies, including large-scale clinical studies, and conducting research during routine clinical practice. In large-scale clinical studies, we have primarily collaborated in subanalyses in the Japanese Investigation of Kinetic Evaluation in Hypertensive Event And Remodeling Treatment (JIKEI HEART) study and in such studies as the Japanese Rhythm Management Trial for Atrial Fibrillation (J-RHYTHM II) study (a multicenter study of upstream drug therapy for atrial fibrillation associated with hypertension comparing calcium antagonists and angiotensin receptor blockers [ARBs]), the Assessment of  $\beta$ -Blocker Treatment in Japanese Patients with Chronic Heart Failure (J-CHF) study (a large-scale clinical study to establish a  $\beta$ -blocker treatment method in chronic heart failure), the Pitavastatin Heart Failure (PEARL) study (a multicenter cooperative study to investigate the ameliorative effect of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on chronic heart failure), and the Nationwide Gender-based Atherosclerosis Determinants Estimation and Ischemic Cardiovascular Disease Prospective Cohort (NADESICO) Study (a multicenter cooperative prospective cohort study of sex differences in risk factors for arteriosclerotic diseases and prevention), which used computed tomographic examinations of coronary arteries.

We have converted patient data, including risk factors and lesion morphology, from catheter examinations and treatment in various clinical research divisions into a database and performed a study comparing risk factors, outcomes, and other variables of ischemic heart disease, cardiomyopathy, and other conditions. In addition, we have participated in nationwide clinical studies (the Japan-Drug Eluting Stents Evaluation: a Randomized Trial [J-DESSERT]; the Coronary Spasm Association [CSA]; Japan Unprotected Left Main Coronary Artery Disease PCI Strategy on New Generation Stents [J-LESSON]; and Omega-3 Fatty Acids for Prevention of Post-operative Atrial Fibrillation [OPERA] trial), mainly investigating in detail treatment with drug-eluting stents and the diagnosis of coronary vasospasm, which is closely related to the etiology of ischemic heart disease.

In regard to heart failure, which is an extremely common form of circulatory pathology,

we have been assessing data related to the concentration of serum brain natriuretic peptide, which is an index of circulatory pathology, and been conducting research on standard values that will be of use in clinical practice. In addition, we have reported in detail the pathology of heart failure before and after admission to the hospital and are now assessing clinical data that will serve as a new index.

We have been aggressively treating atrial fibrillation by catheter ablation, and during this fiscal year we have treated approximately 180 patients. In addition, in clinical research we have investigated 1) the usefulness of the pulmonary vein antrum isolation procedure by new mapping systems, and 2) optimal catheter ablation strategies for persistent atrial fibrillation.

### *Basic research*

Research activity, such as studies at other institutions in Japan and abroad by graduate students in basic sciences and clinical sciences and presentation of the results of many studies, is being performed. In the field of arrhythmias, we have performed research in the form of a study of the basis of the development of atrial fibrillation by using various experimental models in regard to the effects of inflammatory cell invasion and fibrosis on the myocardium. In the field of cardiomyocyte physiology, we have investigated the physiological and pathophysiological regulatory mechanisms of myocardial contraction and relaxation and performed a study with molecular biology techniques and physiological techniques. We have also investigated a new signal transmission system in the  $\alpha$ -receptor stimulation effect in relation to L-type Ca channels in the rat myocardium, the effect of  $\beta$ -receptor stimulation in sarcoplasmic reticulum function, and cardiomyocyte intracellular Ca kinetics in mice in which dilated cardiomyopathy develops because of troponin T mutations. In the field of myocardial metabolism, we have investigated the association between ischemia-reperfusion damage and intracellular ion kinetics in isolated perfused hearts of mice with type 2 diabetes.

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## Department of Internal Medicine

### Division of Diabetes, Metabolism and Endocrinology

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Kazunori Utsunomiya, *Professor*  
 Junichi Yokoyama, *Professor*  
 Katsuyoshi Tojo, *Professor*  
 Yutaka Mori, *Professor*  
 Masami Nemoto, *Associate Professor*  
 Rimei Nishimura, *Associate Professor*

Yoichi Sakamoto, *Professor*  
 Takashi Sasaki, *Professor*  
 Kuninobu Yokota, *Professor*  
 Hideaki Kurata, *Associate Professor*  
 Tamotsu Yokota, *Associate Professor*  
 Shuichi Kato, *Assistant Professor*

### General Summary

Physicians should aim 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*

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 interventional study of childhood obesity and glucose intolerance has also continued. Several clinical trials of the treatment of type 2 diabetes using continuous glucose monitoring (CGM) are under way.

#### *Genetics*

**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 glycconeogenic enzyme is inactive. Therefore we focused on key glycconeogenic 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 the same mutant genotype of compound heterozygosity for *FBPI* (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 *FBPI* 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 receiving tube feeding a highly monounsaturated enteral formula was shown with CGM to suppress postprandial hyperglycemia and to reduce 24-hour glycemic variations to greater extents compared with a high-carbohydrate enteral 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 regulates both microangiopathy and macroangiopathy could lead to novel therapeutic strategies against diabetic complications. The Rho GTPases 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 used db/db mice to study further mechanisms by which ROCKs regulate diabetic macroangiopathy. We found that ROCKs induce expression of monocyte chemoattractant protein 1 through activation of p38 mitogen-activated protein kinase and nuclear factor  $\kappa$ B under diabetic conditions. Furthermore, we found that ROCKs regulate the expression and function of hypoxia-inducible factor 1 $\alpha$ , thereby inducing glomerulosclerosis under diabetic conditions. Finally, we established a primary culture system for Schwann cells from diabetic mice.

## Endocrinology

To identify and separate 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 cardiomyocyte culture system.

Previous studies have shown that the secretion of adrenocorticotrophic hormone is increased in the hearts of patients with hypertension, indicating that adrenocorticotrophic 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.

## Publications

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**Nishimura R, Nakagami T, Sone H, Ohashi Y, Tajima N.** Relationship between hemoglobin A1c and cardiovascular disease in mild-to-moderate hypercholesterolemic Japanese individuals: subanalysis of a large-scale randomized controlled trial. *Cardiovasc Diabetol.* 2011; **10**: 58.

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**Hosoya T, Matsushima M, Nukariya K, Utsunomiya K.** The relationship between the severity of depressive symptoms and diabetes-related emotional distress in patients with type 2 diabetes. *Intern Med*. 2012; **51**: 263-9.

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obese patients with hyperinsulinemia assessed by oral glucose tolerance test (ATHLETE). *Adv Ther*. 2011; **28**: 698-706.

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## Department of Internal Medicine

### Division of Clinical Oncology/Hematology

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 Daisuke Inoue, *Associate Professor*  
 Hidekazu Masuoka, *Assistant Professor*  
 Kaichi Nishiwaki, *Assistant Professor*  
 Yuhichi Yahagi, *Assistant Professor*  
 Yoji Ogasawara, *Assistant Professor*

Tadashi Kobayashi, *Professor*  
 Noriko Usui, *Associate Professor*  
 Takaki Shimada, *Assistant Professor*  
 Nobuaki Dobashi, *Assistant Professor*  
 Shingo Yano, *Assistant Professor*  
 Yutaka Takei, *Assistant Professor*  
 Katsuki Sugiyama, *Assistant Professor*

#### General Summary

The immediate goals of our clinical and basic research are to investigate basic and clinical aspects of malignant diseases and to try to improve outcomes for patients with solid tumors and hematological malignancies, leading to the ultimate goals of improving the natural history of malignant diseases. We have also been performing several clinical trials and basic research studies throughout 2011.

#### Research Activities

##### *Leukemias*

Many patients with previously untreated hematological disorders have been referred to our department. The disorders in 2011 included acute myeloid leukemia (AML), 20 cases; acute lymphoblastic leukemia (ALL), 4 cases; chronic myeloid leukemia (CML), 5 cases; and myelodysplastic syndrome (MDS), 9 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 AML, ALL, and CML. The JALSG protocol studies performed in 2011 were as follows: AML/MDS-HR CS-7 study of newly diagnosed AML; refractory anemia with excess blasts II, all-case registration cohort study; APL-204 (phase III); CML-207 (phase III); AML-209-GS; and AML209-KIT. 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 2011 we registered 69 patients with newly diagnosed non-Hodgkin's lymphoma and 1 patient 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 (bi-R-CHOP±CHASER vs

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). A study of enzastaurin (non-Hodgkin's lymphoma: phase III double-blind) has been completed. Enzastaurin is a novel drug targeting protein kinase C $\beta$  which has been extensively studied throughout the world, including in the United States, the European Union, and Japan.

### *Myeloma*

We registered 13 patients with newly diagnosed multiple myeloma in 2011. 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.

### *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, 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 FOLFIRI have also been performed.

### *Palliative care*

The mission of the palliative care team for cancer pain is to relieve patients' pain and anxiety to support the fight against cancer. Our team encourages the use of narcotics and has improved the control of cancer pain. In our division, we aim to attain individual goals by sharing our thoughts and to contribute to the further growth of palliative care at The Jikei University Hospital.

### *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 gammopathy 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-positive cells allows us to try such a strategy. Growth and differentiation of CD34-positive cells into which the *MDR1* gene has been transferred has been investigated *in vitro* in collaboration with Keio University's Department of Pharmacy. The results have recently been published, and further research is in progress.

## **Publications**

**Inoue D, Sakuyama T, Ichiba T, Uwagawa T, Arakawa Y, Nagasaki E, Yoshizawa A, Matsumoto T, Aiba K.** Usefulness of epidural catheter with the subcutaneous reservoir for cancer pain control in homecare service (in Japanese). *Gan to Kagaku Ryoho*. 2011; **38** Suppl 1: 61-3.

**Arakawa Y, Inoue D, Sakuyama T, Nagasaki E, Aiba K.** Strontium-89 therapy and subarachnoid phenol block successfully eliminated intractable pain of metastasis in the patient with advanced urachal carcinoma (in Japanese). *Gan to Kagaku Ryoho*. 2011; **38** Suppl 1: 59-60.

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**Yano S, Minami J, Nishiwaki K, Shimada T, Dobashi N, Yahagi Y, Takei Y, Takahara S, Ogasawara Y, Sugiyama K, Yamaguchi Y, Saito T, Kasama K, Yokoyama H, Machishima T, Katsube A, Usui N, Aiba K.** Rapid progression and unusual premortal diagnosis of mucormycosis in patients with hematologic malignancies: analysis of eight patients. *Int J Hematol*. 2011; **93**: 344-50.

## Department of Internal Medicine

### Division of Respiratory Diseases

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Kazuyoshi Kuwano, *Professor*  
Katutoshi Nakayama, *Associate Professor*  
Jun Araya, *Assistant Professor*

Akira Kojima, *Associate Professor*  
Masamichi Takagi, *Assistant Professor*

#### General Summary

We have performed clinical and basic research concerning chronic obstructive pulmonary disease (COPD), bronchial asthma, pulmonary infection, pulmonary fibrosis, and lung cancer. Basic research should resolve clinical problems, and clinical research should lead to novel treatments. We completed clinical research concerning COPD in collaboration with the Department of Cardiology and the Department of Diabetes, Metabolism and Endocrinology. Basic research focused on the molecular mechanisms of lung injury, fibrosis, and COPD are progressing. We investigated the roles of apoptosis, senescence, and autophagy in the pathogenesis of various lung diseases.

#### Research Activities

##### *COPD*

Clinical research concerning the incidence of COPD in patients with diabetes mellitus, coronary artery disease, or heart failure has been completed. Serum levels of proinflammatory cytokines, such as tumor necrosis factor, interleukin (IL) 1, and IL-6 were measured in these patients. Oxidative stress in patients with COPD was estimated by measuring urine levels of 8-hydroxydeoxyguanosine. The effect of steroid inhalation on oxidative stress in patients with COPD has been investigated. We hypothesized that early intervention against COPD can prevent various comorbidities. We found that the prevalence of COPD was higher in patients with coronary artery diseases, heart failure, or diabetes mellitus than in control subjects. Serum levels of tumor necrosis factor and C-reactive protein were decreased in patients treated with statins. Urine levels of 8-hydroxydeoxyguanosine in patients with COPD were higher than in other subjects. We are performing clinical research to examine the effects on comorbidities of treatments for COPD, cardiovascular diseases, and diabetes mellitus. We will soon analyze the effects of intervention for 1 year.

##### *Infection and lung injury*

Double-stranded RNA viruses are associated with acute lung injury. We investigated the effect of insulin on epithelial cell fate after damage by polyinosinic-polycytidylic acid. Our studies with human bronchial epithelial cells in primary culture found that insulin was required to protect these cells from apoptosis induced by polyinosinic-polycytidylic acid. Apoptotic signals were dependent on activation of caspase 8. We also found that survival signals were mainly through ERK and AKT activation, although other

survival signals may also be associated. We suggest that insulin administration is a promising strategy against acute lung injury induced by viral infection. These results were published in *Journal of Immunology*. We are also investigating the mechanisms of lung injury in influenza virus pneumonia.

#### *Idiopathic pulmonary fibrosis*

Aberrant re-epithelialization with bronchial epithelial cells is a prominent pathologic finding in idiopathic pulmonary fibrosis (IPF) and is implicated in abnormal epithelial-mesenchymal interactions. Recent studies show that senescence is a risk factor for development of IPF. Among the sirtuin (SIRT) family of class III histone deacetylases, SIRT6 has been demonstrated to antagonize senescence. We examined epithelial senescence as a representative phenotypic alteration in conjunction with SIRT6 expression in IPF. We have obtained 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 beta (TGF- $\beta$ ) induces senescence by increasing p21 expression and also induces SIRT6 expression, and artificial overexpression of SIRT6 efficiently inhibits TGF- $\beta$ -induced senescence via proteasomal degradation of p21. Secretion of IL- $\beta$ 1 from human bronchial epithelial cells induced by TGF- $\beta$  to become senescent is responsible for myofibroblast differentiation in fibroblasts. These findings shed light on the accelerated epithelial senescence in IPF and on a possible regulatory role for SIRT6. These results were published in *American Journal of Physiology Lung Cell and Molecular Physiology*. We are also investigating the role of autophagy in IPF.

#### *Autophagy in bronchiolar epithelial cells*

To investigate the significance of autophagy in lung diseases, we examined the association between autophagy and senescence in bronchial epithelial cells. Cigarette smoke extract (CSE) induced senescence in bronchial epithelial cells. Autophagy in these cells was transiently upregulated by CSE but was then downregulated by CSE. Furthermore, CSE increased miss-folded and ubiquitinated proteins and induced senescence in these cells. Autophagy digested these unnecessary proteins and protected these cells from senescence. We suggest that autophagy plays important roles in maintaining homeostasis in lung epithelial cells. These results were published in *Oncoimmunology* (2012).

#### *Lung cancer*

Clinical research about the effects of nitroglycerin on chemotherapy in non-small cell lung cancer is going. This study is a multicenter trial in Japan. A study of the role of endothelial progenitor cells in the progression and treatment of lung cancer is being planned.

#### **Publications**

**Numata T, Araya J, Fujii S, Hara H, Takasaka N, Kojima J, Minagawa S, Yumino Y, Kawaishi**

**M, Hirano J, Odaka M, Morikawa T, Nishimura SL, Nakayama K, Kuwano K.** Insulin-depen-

dent phosphatidylinositol 3-kinase/Akt and ERK signaling pathways inhibit TLR3-mediated human bronchial epithelial cell apoptosis. *J Immunol.* 2011; **187**: 510-9.

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**Fujita Y<sup>1</sup>, Hirano S<sup>1</sup>, Takeda Y<sup>1</sup>, Izumi S<sup>1</sup>, Ishii S<sup>1</sup>, Naka G<sup>1</sup>, Iikura M<sup>1</sup>, Kobayashi N<sup>1</sup>, Kudo K<sup>1</sup> (<sup>1</sup>Natl Ctr Global Health Med Jpn).** Bronchoscopic comparison of pulmonary complications arising in patients with and without hematopoietic stem cell transplantation (in Japanese). *Kikanshigaku.* 2011; **33**: 241-9.

## Reviews and Books

**Ishikawa T, Nakayama K.** Mechanisms and measures of COPD exacerbation (in Japanese). *Kokyukinaika.* 2011; **20**: 215-22.

**Kuwano K, Araya J, Hara H.** Aging and interstitial lung diseases (in Japanese). *Kokyu to Junkan.* 2011; **59**: 577-85.

## Department of Internal Medicine

### Division of General Medicine

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Tatsuo Hosoya, *Professor*  
 Norio Tada, *Professor*  
 Masami Nemoto, *Associate Professor*  
 Nobuyuki Furutani, *Associate Professor*  
 Mie Kawai, *Assistant Professor*  
 Takanori Ebisawa, *Assistant Professor*

Nobuakira Takeda, *Professor*  
 Akihiro Nishiyama, *Associate Professor*  
 Hiroshi Yoshida, *Associate Professor*  
 Jun Hiramoto, *Associate Professor*  
 Chihiro Shikata, *Assistant Professor*

#### Research Activities

##### *Division of General Medicine, The Jikei University Hospital*

We are attempting to compile a database of our medical examinations and treatments during primary care in outpatient units. The data and information of every outpatient are gathered from forms of our own design filled out by the physicians. The data and information include reason for visiting, physical symptoms and complaints, whether the patient had consulted other physicians, the primary diagnosis, examinations, and care. The data we compile, especially from initial visits, are expected to be useful for analyzing trends in primary care at large general hospitals.

##### *Division of General Medicine, The Jikei University Aoto Hospital*

Biochemical and molecular biological examinations were performed of rats with an experimental model of heart failure in collaboration with a Canadian research group. Myocardial subcellular changes were investigated. Histochemical examination of heart failure was also performed in collaboration with a German research group. The effects of sarpogrelate, a 5-hydroxytryptamine (2A) receptor antagonist, were examined following the project of last year.

##### *Division of General Medicine, The Jikei University Daisan Hospital*

###### 1. Study of factors of infection in elderly hospitalized patients

We studied the relations of infection to nutritional status, administered drugs, and biochemical markers. We found that the poor nutritional status and the use of gastric acid-suppressing drugs promote infection in elderly hospitalized patients.

###### 2. Study of tube feeding

We attempted to determine the best method of tube feeding of elderly patients.

###### 3. Study of the relations of severe infection to electrolyte abnormality and hormonal abnormality

We studied the relations of severe infection to hypophosphatemia and hypothyroidism.

###### 4. Early diagnosis of severe sepsis

Severe sepsis has a high mortality rate. We studied several variables in severe sepsis. Procalcitonin is useful for diagnosing severe sepsis due to Gram-negative rods.

*Division of General Medicine, The Jikei University Kashiwa Hospital*

Our research at Kashiwa Hospital has 3 goals. The first is to investigate the role of general medicine on environmental health achievement in the areas, especially in Kashiwa. We also participated again this year in the development of the local health care system in Kashiwa as a member of the local governance committee. The second goal is to evaluate abnormalities of lipids and lipoproteins which contribute to premature atherosclerosis and to develop treatments for atherosclerotic disorders. The effects of co-feeding with carbohydrates and lipids on postprandial hyperlipidemia were investigated by measuring serum levels of apolipoprotein B48 level; our results were reported at the XVI<sup>th</sup> International Symposium on Atherosclerosis held in Sydney, Australia. The third goal is to develop educational tasks for teaching medical students and junior physicians.

### Publications

**Hosoya T, Matsushima M, Nukariya K, Utsunomiya K.** The relationship between the severity of depressive symptoms and diabetes-related emotional distress in patients with type 2 diabetes. *Intern Med.* 2012; **51**: 263-9.

### Reviews and Books

**Tada N, Maruyama C, Koba S, Tanaka H, Birou S, Teramoto T, Sasaki J.** Japanese dietary lifestyle and cardiovascular disease. *J*

*Atheroscler Thromb.* 2011; **18**: 723-34.

**Koba S, Tanaka H, Maruyama C, Tada N, Birou S, Teramoto T, Sasaki J.** Physical activity in the Japan population: association with blood lipid levels and effects in reducing cardiovascular and all-cause mortality. *J Atheroscler Thromb.* 2011; **18**: 833-45.

**Hosoya T, Matsushima M.** *Evidence Update* 2012, therapeutic agents of diabetes, Metformin (in Japanese). *Yakkyoku.* 2012; **63**: 52-6.

## Department of Psychiatry

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Kazuhiko Nakayama, *Professor*  
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 Hironari Sue, *Associate Professor*  
 Wataru Yamadera, *Assistant Professor*  
 Kazuya Ono, *Assistant Professor*  
 Rieko Shioji, *Assistant Professor*  
 Tatsuhiko Itoh, *Assistant Professor*  
 Norifumi Tsuno, *Assistant Professor*

Hiroshi Itoh, *Professor*  
 Hisatsugu Miyata, *Professor*  
 Kazutaka Nukariya, *Associate Professor*  
 Motohiro Ozone, *Assistant Professor*  
 Keita Ohbuchi, *Assistant Professor*  
 Ayumi Tateno, *Assistant Professor*  
 Koji Nakamura, *Assistant Professor*

### General Summary

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

### Research Activities

#### *Psychopathology, psychotherapy, and the child study group*

We have promoted research in the fields of psychotherapy, psychopathology, and child psychiatry. We have been investigating the care systems for developmental disorders in the psychiatry unit. We then began to study the attention problems of developmental disorders and psychotic disorders. This study investigated the quality of attention maintained in autism-spectrum disorders, but when many tasks were added, the quality of attention tended to decrease.

In the field of psychotherapy, we have tried to develop a prototype of dialectical behavior therapy for Japanese, diary therapy, and the Self-Psychological Psychotherapeutic Approach, which maintains the self-esteem of patients with developmental disorders.

Our social psychiatry team has 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 become exhausted when trying to cope with complex human relationships in the workplace. Female workers having the double burden of family commitment and perfectionism tend to be isolated in terms of personal relationships, leading to exhaustion both inside and outside the workplace.

#### *Morita therapy group*

Based on the “Guideline for Outpatient Morita Therapy” we started to develop a standard package of outpatient Morita therapy and a proper method for assessing the outcome of this treatment. Studies that have been continued this year include those on the relationship between panic disorder and generalized anxiety disorder, the subtypes of obsessive-compulsive disorder, “fall-in reaction” occurring in the process of mood or anxiety disorders, and the recovery factors of inpatient Morita therapy for patients with depression. A qualitative study of the life histories of female patients with obsessive-compulsive disorder has also been performed. In addition, we have been continuously promoting com-

parative studies between Morita therapy and the third generation of cognitive-behavioral therapies, such as acceptance and commitment therapy.

#### *Psychopharmacology group*

In basic research we studied: 1) the mechanisms of the central nervous system actions of a new generation of psychotropic drugs were studied with microdialysis and radioimmunoassay and 2) the brain mechanisms underlying drug dependence, impulsivity related to dependence, and next-generation medicines for drug dependence (in collaboration with NTT Communication Science Laboratories and Senshu University). In clinical research, we studied: 1) the effectiveness of second-generation antipsychotic drugs on anxiety and stress-related disorders, 2) the involvement of the medial prefrontal cortex in motivation-oriented behavior (functional magnetic resonance imaging study in collaboration with the National Institute of Radiological Science), 3) nerve growth factors involved in neurodegenerative diseases (genetic research in collaboration with the Institute of DNA Medicine), 4) genetic factors related to the therapeutic mechanism of modified electroconvulsive therapy, and 5) symptoms of mental disorders related to menstruation, atypical psychosis, and acute psychosis. Integration of basic and clinical research is a fundamental concept of the psychopharmacological group.

#### *Psychophysiology group*

Studies examined: 1) changes in sleep structures and cognitive function according to the menstrual cycle by means of the cyclic alternating pattern method, 2) multicenter research regarding the development of a novel platform on websites for sleep medicine and research, 3) the development of skills for diagnosing and treating insomnia, 4) the efficacy of group cognitive behavioral therapy for insomnia for chronic sleep disturbance and depression, 5) the Multiple Sleep Latency Test in hypersomnias of central origin.

#### *Psychogeriatric group*

First, a study of the neuropsychological evaluation of neurodegenerative disorders using brain imaging modalities, such as magnetic resonance imaging and single-photon emission computed tomography, which suggested that a reduction in hippocampal volume in Alzheimer's disease is related to delayed responses on neuropsychological tasks. Second, an epidemiological survey conducted in Itoigawa City showed no differences in mortality rates in patients with dementia, although treatment was more costly under the Long-Term Care insurance system for patients with vascular dementia than for patients with Alzheimer disease. Third, a longitudinal study of the prevalence of psychiatric disorders in patients with breast cancer was performed in collaboration with the Department of Surgery.

#### *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 patients with insomnia was performed. Another study investigated the issues associated with mental care services for patients with cancer and for their families and medical staff.

#### *Clinical electroencephalography group*

We examined the safety and efficacy of psychotropic drugs in several forms of psychosis associated with epilepsy. We reported on 2 patients with epilepsy and reflex seizures caused by psychological state. Furthermore, we discussed the characteristics of falls in patients with epilepsy and of dental and orofacial injuries in patients with cognitive disabilities. A study was performed to prevent the recurrence of depression in patients with epilepsy.

#### *Clinical psychology group*

We have continued to discuss and study psychotherapeutic processes and the treatment techniques of cognitive behavior therapy, Morita therapy, relief care, psycho-oncology, and mental care after natural disasters. We have also examined the characteristics of developmental disorders and higher brain dysfunctions through psychological assessments. We invited Ms.Kayo Akashi to a clinical conference on skills for psychological recovery (We could not invite her last year because of the Great East Japan Earthquake), and studied more practical mental support after disasters. Furthermore, we trained graduate students of a clinical psychological course.

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## Department of Pediatrics

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Hiyoruki Ida, *Professor*  
 Ichiro Miyata, *Associate Professor*  
 Yasuyuki Wada, *Associate Professor*  
 Kazue Saito, *Associate Professor*  
 Hiroshi Kobayashi, *Associate Professor*  
 Masako Fujiwara, *Assistant Professor*  
 Masaharu Akiyama, *Assistant Professor*  
 Takashi Urashima, *Assistant Professor*

Tohya Ohashi, *Professor*  
 Toshio Katsunuma, *Associate Professor*  
 Yoko Kato, *Associate Professor*  
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 Yoshihiro Saito, *Assistant Professor*  
 Hiroshi Tachimoto, *Assistant Professor*  
 Masahisa Kobayashi, *Assistant Professor*

### General Summary

We have 9 subspecialty research groups: the Inherited Metabolic Disease, Endocrinology, and Medical Genetics 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 provide practical benefits to patients and their families through basic and translational research and clinical study.

### Research Activities

#### *Inherited Metabolic Disease, Endocrinology, and Medical Genetics group*

Accomplishments of our group this year are follows.

1. We clarified the mechanism of the build-up of autophagy in Pompe disease cells and developed a novel method to control autophagy.
2. We found that proteasome inhibitors improve the function of mutant lysosomal alpha-glucosidase in fibroblasts from a patient with Pompe disease.
3. We clarified the effect of antibodies against an infused enzyme in enzyme-replacement therapy for Fabry disease.
4. We developed a novel gene therapy approach using a lentiviral vector.
5. We analyzed the expression in the brain of urocortin 2, urocortin 3, corticotropin-releasing factor receptor 2, and nesfatin 1 in a rat model of left ventricular failure.
6. We studied insulin insufficiency in Rota virus infection.

By using a comparative genomic hybridization array for genome-wide screening, we have detected submicroscopic pathogenic genome imbalances with a diagnostic yield of 12% in patients with congenital malformations.

#### *Neurology group*

1. We investigated the development of posttraumatic epilepsy in 142 children with sequelae of traumatic brain injury. Epilepsy developed in 37 patients. The risk factors for posttraumatic epilepsy were child abuse, acute subdural hematomas, severe and long-term loss of consciousness, and such complications as poor mobility and mental deterioration.
2. We evaluated the efficacy of intravenous antiepileptic drugs for treating status epilep-

ticus in children. Of the 189 episodes of status epilepticus, 42.3% were in children with epilepsy and 41.3% were febrile seizures. The most frequently administered agents were diazepam as a first-line treatment and intravenous midazolam or phenobarbital as second-line treatments. Phenobarbital and diazepam were effective in at least 70% of cases. Thiopental was the agent most likely to produce adverse effects. Diazepam as a first-line treatment and phenobarbital and intravenous midazolam as second-line treatments may be practical for status epilepticus in children.

#### *Hematology and Oncology group*

Telomerase, a ribonucleoprotein DNA polymerase that elongates the telomeres of chromosomes to compensate for losses during DNA replication, is constitutively expressed in most malignant tumor cells. We have established a human megakaryocytic-erythroid cell line that expresses the erythropoietin receptor. Erythropoietin is a hematopoietic growth factor that regulates cellular proliferation and differentiation in the erythroid lineage. However, the mechanism by which telomerase regulation is modulated by erythropoietin has remained unclear. We demonstrated that erythropoietin activates telomerase in JAS-REN-A cells through dual regulation: *hTERT* gene transcription by JAK2/STAT5/c-Myc and hTERT protein phosphorylation by phosphatidylinositol 3'-kinase/AKT.

Pediatric palliative care programs for children with cancer in university hospitals have unique challenges. We established a pediatric palliative care education program in the undergraduate medical course.

#### *Infectious Diseases and Immunologic Disorders group*

We focus on the identification of causative pathogen by means of polymerase chain reaction techniques, genetic diagnosis and treatment of primary immunodeficiency syndrome, and analysis of immune response in pediatric rheumatic diseases.

Our research and development achievements were as follows.

1. Rapid identification of causative pathogens in inflammatory diseases using multiplex polymerase chain reaction

We showed that our assay was more sensitive, more specific, and faster than the gold-standard culture-based method.

2. *Ex vivo* gene therapy for X-linked chronic granulomatous disease

We submitted an application to perform a clinical trial of retroviral gene therapy for X-linked chronic granulomatous disease.

3. Fecal microbial composition of healthy infants and patients with chronic granulomatous disease

We found that in patients with chronic granulomatous disease that the populations of *Bacteroides* and *Bifidobacterium* were significantly smaller in patients with enteritis than in patients without enteritis.

#### *Nephrology*

We investigated the clinical characteristics and the efficacy of voiding cystourethrography (VCUG) in infants with febrile urinary tract infection (UTI).

We analyzed the medical records of 115 infants (mean age,  $2.84 \pm 2.18$  months; male:female ratio, 83:32) who were admitted to the hospital with febrile UTI from January 1999 through May 2009. A UTI was diagnosed when a bacterial quantitative culture of catheter-obtained urine was more than  $10^4/\text{ml}$ , or  $10^3/\text{ml}$  when clinical characteristics and laboratory data were considered. We performed VCUG for boys at the first UTI and for girls at the second UTI.

When urinary organ anomalies, such as vesicoureteral reflux (VUR), were detected with VCUG, we consulted pediatric urologists and continued to follow up the cases.

The bacteria most often identified with urine culture were *Escherichia coli* (n=83) and *Enterococcus* (n=28). A total of 75 patients, of whom 70 were boys, underwent VCUG. Thirteen patients had VUR, and 5 patients had other anomalies. Of these 18 patients, 8 (5 with VUR and 3 with other anomalies) required surgery. Neither the clinical characteristics nor laboratory data differed significantly between cases with or without abnormalities identified with VCUG. In conclusion, VCUG is useful for diagnosing operation adaptive anomalies, including VUR.

### Cardiology

The Pediatric Cardiology group is interested in both basic and clinical cardiology research to improve the outcomes of children with congenital heart issues. The results of our research have been presented at the annual meetings of the Japan Pediatric Society and the Japan Pediatric Cardiology Society. Our research projects on right ventricle heart failure and copy number variants in congenital heart disease received funding from the Japanese society for the promotion of science. Specific projects under way in our group are as follows.

1. The effect of telmisartan in right-heart failure
2. Cardiac apoptosis in right-heart failure
3. Clinical outcomes of cardiac morphology involved in congenital metabolic disorders
4. Gene transmutation in patients with noncompunction

We also are interested in clinical research, specifically:

1. A management of fetal cardiac issues
2. Long-term outcomes in patients with total cavopulmonary connection circulation
3. Interventional catheterization (balloon angioplasty and valvuloplasty, coil embolization, transcatheter stenting, and catheter closure of congenital heart defects)

### Allergy

The main subjects of our research are as follows: 1) the role of eosinophils and mast 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:

PET study: Preventive effect of tulobuterol patch for the long-term management of infantile asthma study

PARG study: Pediatric Asthma Research for Guideline Update: Add-on use of tulobuterol patch for unstable asthma treated with leukotriene receptor antagonist

CIT study: A comparison of continuous inhalation treatment with salbutamol and isopro-

terenol for severe pediatric bronchial asthma: A multicenter, double-blind, randomized study

OSCAR study: Optimal stepdown therapy for controlled pediatric asthma responding to salmeterol/fluticasone

ORIMA study: Effect of oral immunotherapy in preschool children with milk allergy

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## Department of Dermatology

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Mariko Honda, *Professor*  
Arihito Ota, *Associate Professor*  
Masaaki Kawase, *Associate Professor*  
Yoshinori Umezawa, *Assistant Professor*

Ryoichi Kamide, *Professor*  
Takaaki Ishiji, *Associate Professor*  
Hidehisa Saeki, *Associate Professor*  
Toshihiro Ito, *Assistant Professor*  
Koma Matsuo, *Assistant Professor*

### General Summary

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

### Research Activities

#### *Psoriasis*

Various systemic therapies, including oral cyclosporin microemulsion concentrate, methotrexate, etretinate, topical vitamin D3, and corticosteroids, have been used, depending on disease severity and the degree to which quality of life (QOL) is impaired in individual patients. Also phototherapy, including psoralen ultraviolet A, narrow-band ultraviolet B (UVB), and the 308-nm excimer lamp, are effective and have been administered in a newly organized skin-care clinic. 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 intractable psoriasis. Clinical trials have been performed with new biologic agents, including antibodies against interleukin (IL) 17A, IL-23p19, and janus kinase 1/3 inhibitor.

#### *Atopic dermatitis*

The pathogenesis of atopic dermatitis has been attributed to a complex interaction of the environment, 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 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 learn how QOL is impaired. To support this type of approach, we have organized skin-care lessons in the Skin-Care Clinic twice a week and have hosted an atopic dermatitis forum, which includes monthly lectures and group meetings. For basic clinical research, the levels of substance P, thymus and activation-regulated cytokine, and IL-31 related to pruritus in atopic dermatitis are being evaluated according to disease

severity. Clinical trials of opioid- $\kappa$ -receptor 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, malignant peripheral nerve sheath tumor, malignant fibrous tumors, and cutaneous T-cell lymphomas. For the accurate diagnosis of pigmented tumors, we always perform dermoscopic examinations and sentinel lymph-node biopsy, especially for patients with stage II or III melanoma. We are participating in collaborative clinical research for maintenance therapy using local injections of interferon  $\beta$  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 malignant peripheral nerve sheath tumor (MPNST) in patients with neurofibromatosis 1 is 10%, although information concerning the epigenetic abnormality is limited. We have used the methylation-specific polymerase chain reaction (PCR) and real-time reverse transcriptase (RT)-PCR to analyze the methylation status of tumor suppressor genes and cancer-testis genes in established MPNST cell lines. 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 variciclovir are started to improve the impaired QOL.

#### 2. Herpes zoster and postherpetic neuralgia

Initial treatments for herpes zoster and postherpetic neuralgia (PHN) are performed in this clinic. 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. Posthoc analyses of a subgroup of patients showed that amitriptyline in combination with acyclovir reduced 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 antide-

pressants, selective serotonin reuptake inhibitors, opioid analgesics, such as Tramcet<sup>®</sup> (Grunethal Ltd., Stokenchurch, UK), which contains tramadol hydrochloride and acetaminophen. Tramadol is a weak  $\mu$ -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, topical vitamin D3 and salicylic acid have been used to treat viral warts. Contact immunotherapy using squaric acid dibutylester, CO<sub>2</sub> laser, pulsed dye laser, and glutaraldehyde 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 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 treatment is not covered by health insurance, so 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<sub>2</sub> 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, 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 introduced. New biologic agents, including infliximab, adalimumab and ustekinumab, have been also 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 inpatients and outpatients to enhance QOL. Genetic analysis was performed for MPNST.

**Herpes virus infection:** Suppressive therapy has been used to improve impaired QOL. Surveys of QOL in patients with recurrent genital herpes and drug sensitivities derived from HSV are also being performed. To control PHN, we are prescribing tricyclic antidepressants, serotonin reuptake inhibitors, Tramcet® and other opioid analgesics, and topical analgesics.

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

**Contact dermatitis:** Causal chemicals, environmental allergens, drugs, and foods in patients with contact dermatitis, are regularly performed patch testing.

**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, Th2 chemokines, and thymus and activation-regulated cytokine.

**Malignant skin tumors:** We have been treating many patients with skin cancers, including melanomas, basal/squamous cell carcinoma, 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 QOL for patients with incurable conditions.

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

**Collagen vascular diseases:** Intimate and periodic follow-up is performed in cooperation with other departments.

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

### **Publications**

**Fujita Y, Tsunemi Y, Kadono T, Saeki H, Mori E, Le Pavoux A, Watanabe T, Kikuchi K, Tamaki K.** Lipidized fibrous histiocytoma on the left condyle of the tibia. *Int J Dermatol.* 2011; **50**: 634-6.

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## Department of Radiology

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 Takuji Mogami, *Assistant Professor*

### Research Activities

#### *Division of diagnostic imaging*

1. Computed tomography scoring system as a predictor of metastasis to the neck 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 comprises the 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. Morphological and hemodynamic evaluation of the cardiovascular system with dual-source CT

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

4. Imaging ovarian borderline tumors

The CT and magnetic resonance (MR) findings of ovarian borderline tumors (OBTs) were assessed. The appearance of OBTs differs between histological types. They often mimic malignant tumors, with such CT and MR features as solid portions and wall thickening. Calcifications are often present. Representative histological types of OBTs are serous and mucinous tumors. Serous tumors tend to be smaller than mucinous tumors. They usually have solid portions and show characteristic papillary projections. Mucinous tumors are usually large and multicystic. They often have localized wall thickenings or small solid components or both.

5. Usefulness of contrast-enhanced MR for evaluating the therapeutic effects of biological agents against tumor necrosis factor  $\alpha$  for psoriatic arthropathy

MR 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 psoriatic arthropathy. In patients with active psoriatic arthropathy, the contrast-enhancement effect was present in enthesitis and the synovitis. These contrast-enhance-

ment effects disappeared where good therapeutic effects were obtained. Contrast-enhanced MR is useful for evaluating therapeutic effects in patients with psoriatic arthropathy.

#### 6. Retro-odontoid soft tissue thickness measured with cervical spine MR

The relationship between retro-odontoid soft-tissue thickness (RSTT) and patients' age and sex and degenerative changes of the cervical spine were analyzed with MR of the cervical spine. Increased RSTT was associated with age, degeneration of the cervical spine, and a history of long-term dialysis. These results suggest that cervical spine degeneration and instability cause retro-odontoid pseudotumor formation.

### *Division of Ultrasound*

#### 1. Clinical usefulness of sonographic contrast examination of breast tumors

The efficacy and safety of ultrasonography with contrast enhancement using Sonazoid microbubbles 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 bilateral wrists, elbows, shoulders, knees, and ankles of patients with rheumatoid arthritis. The synovial blood flow signals were scored with a 3-grade scale, and the total 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. Usefulness of the fractional uptake method and cerebral blood-flow scintigraphy to determine the quantity of cerebral blood flow in childhood

Quantifying cerebral blood flow with cerebral blood-flow scintigraphy in childhood is difficult. We assessed the usefulness of the fractional uptake method for determining the quantity of cerebral blood flow with a whole-body scan. In patients with low mean blood flow less than 20 ml/100 g/minute, the quantitative value determined with the fractional uptake method was higher than the value determined with autoradiography or the graph-plot method. In other patients, the quantitative values obtained with the fractional uptake method correlated well with values obtained with other methods.

#### 2. Physiological changes in the 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. We compared 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 for analyzing regions of interest. In neonates, physiological accumulation was low throughout the brain and was lowest in the frontal lobe. As infants aged, accumulation in all regions increased, especially in the occipital lobe and in the cerebellum. The peak of accumulation was in subjects aged 3 to 17 months. The rate of change in physiological accumulation was least in the

frontal lobe.

#### *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  $46.5\% \pm 31.5\%$ . There was no complications or progressive liver insufficiency after embolization or resection.

#### *Division of Radiation Therapy*

##### 1. Clinical evaluation of re-irradiation for in-field relapse after definitive radiotherapy in head and neck cancer

Because of various morbidities, cure is difficult to obtain after in-field relapse following definitive radiotherapy for head and neck cancer. In cases of solitary recurrence, re-irradiation can be a curative treatment even if the dose is greater than a tolerable level. We have been reviewing cases treated with re-irradiation and are analyzing the efficacy and late complications of re-irradiation.

##### 2. Effect of drug therapy in the induction of bronchiolitis obliterans organizing pneumonia after breast-conserving therapy

Bronchiolitis obliterans organizing pneumonia (BOOP) is a complication of whole-breast irradiation for breast cancer. The incidence of BOOP after radiotherapy is 1.84%, and the interval between radiotherapy and the appearance of BOOP ranges from 2.5 to 9 months. The incidence of BOOP is greater with a longer duration of endocrine therapy, although the relationship is not statistically significant. We have been analyzing the effects of drug therapies, such as chemotherapy, molecularly targeted therapy, and endocrine therapy, on the induction of BOOP.

##### 3. Clinical evaluation of shorter-fraction radiotherapy following breast-conserving therapy

Whole-breast irradiation after breast-conserving therapy is a standard treatment for breast cancer. However, radiation therapy requires a long period of treatment. Hypofractionated radiotherapy requires only 3.5 weeks and can achieve the same degree of local control and the same severity of acute reaction as can standard radiotherapy. We have been evaluating the efficacy of a short course of whole-breast irradiation after breast-conserving therapy.

##### 4. Randomized clinical trial of triple-modality therapy for clinically localized high-risk prostate cancer

For several years we have been treating high-risk prostate cancer with triple-modality therapy. However, the optimal duration of combination neoadjuvant hormonal therapy (NHT) with adjuvant hormonal therapy (AHT) in the setting of high-dose-rate brachy-

therapy (HDR-BT) and external beam radiotherapy has been controversial. To evaluate the efficacy of AHT, we randomly assigned patients with clinically localized high-risk prostate cancer into 2 arms: Arm 1, NHT + HDR-BT + 3-dimensional conformal radiotherapy (3DCRT), and Arm 2, NHT + HDR-BT + 3DCRT + AHT.

#### 5. Computer simulation of skin erythema caused by radiotherapy

We generated a dynamic model (the generalized linear quadratic model) of fractionated radiotherapy that incorporated the “time factor” to simulate the time-dose-fractionation effect on both cancerous and normal tissues. With this model, skin erythema caused by radiotherapy could be demonstrated on a computer display.

#### 6. Database of patients undergoing radiotherapy

To review the structure of radiation oncology in our hospital, a radiation oncology database has been created, with registration starting in June 2011.

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## Department of Surgery

### Division of Digestive Surgery

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Susumu Kobayashi, *Professor*  
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### General Summary

The presentation of study results at annual scientific meetings is not the goal of surgical research. Many abstracts accepted for oral or poster presentations at scientific meetings will go unread and uncited, calling into question the value of the research. Research activities are measured by the quantity and quality of international publications. The impact factor is a surrogate marker for the quality of a journal, and the citation index, which is the number of times an article has been cited, has been used as a marker of its influence in a medical specialty. According to a recent survey in the United Kingdom, only a quarter of abstracts presented at the annual scientific meeting of the British Association of Surgery resulted in peer-reviewed publication with an average impact factor of 1.65 for basic science papers and with an average impact factor of 0.65 for clinical subjects. This survey also indicates that unpublished studies 2 years after presentation will never be published. The number of our abstracts accepted for presentation at Japanese and international medical congresses is increasing, but the number of our publications is not increasing at the same rate. We are encouraged to enhance achievements in academic surgery by improving scientific writing skills.

### Research Activities

#### *Upper gastrointestinal surgery*

We evaluated the pathogenesis of primary esophageal motor functional disorders, especially achalasia, gastroesophageal reflux disease, and reflux esophagitis, using manometry and multichannel intraluminal impedance pH monitoring. We have performed many laparoscopic surgeries and obtained good results. Recently, we began to use a mesh for crural repair in patients with intractable gastroesophageal reflux disease. We aimed to investigate the significance of the expression of small ubiquitin-like modifier 1 as a prognostic molecular marker of esophageal cancer. We continue to assess the viability of the gastric tube using an intraoperative thermal imaging system during esophagectomy to

investigate the correlation between suitable graft reconstruction and postoperative complications. We also continue to examine intraoperative recurrent nerve monitoring to prevent postoperative recurrent nerve palsies and to predict the degree of paralysis after surgery.

We are attempting to optimize the treatment of gastric malignant tumors, such as gastric cancer and gastrointestinal stromal tumor. Our treatment strategies for gastric cancers include intra-abdominal chemotherapy for advanced gastric cancer with possible peritoneal metastasis and sentinel navigation surgery with infrared endoscopy for early gastric cancer. Herceptin was recently approved for the treatment of human epidermal growth factor receptor 2 (HER2)-positive gastric cancer. Because the incidence of HER2-positive gastric cancer has not been extensively studied in Japan, we are studying the positivity rate both retrospectively and prospectively. We have treated 3 patients with recurrence after laparoscopic gastric cancer resection, but the overall long-term prognosis after laparoscopic resection is good.

Postgastrectomy syndrome comprises specific symptoms after gastrectomy and is a target for treatment. The severity of postgastrectomy syndrome is mainly related to the extent of gastric resection and the reconstruction procedures. Postgastrectomy syndrome is a clinical obstacle, because it impairs patients' quality of life. 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.

### *Colorectal surgery*

To improve the quality of laparoscopic operations, we are evaluating the effect of laboratory training with a virtual reality surgical simulator for laparoscopic colectomy. A comparative evaluation of surgical stress between open and laparoscopic colorectal procedures is in progress. In the forthcoming era of robotic surgery for colorectal disease, we are preparing to develop safer and less invasive surgical procedures. In chemotherapy, we are actively participating in national multi-center trials to send new evidence to the world. Moreover, an original regimen are developed in collaboration with the Division of Oncology/Hematology, Department of Internal Medicine, and the characteristic early tumor shrinkage with this regimen has been reported at domestic and foreign congresses. There have been no breakthroughs in basic research on various antibodies in relation to cancer. However, indoleamine 2,3-dioxygenase (an enzyme that mediates cancer immunotolerance) has been reported to be a useful marker for predicting recurrence of early colorectal cancer. In collaboration with the Department of Urology, we are developing a proteomic method to identify cancer-associated proteins (colorectal, esophageal, gastric, pancreatic, and liver cancers). We have started to characterize a novel, targeted, nanobiopolymeric conjugate based on biodegradable, nontoxic, and nonimmunogenic poly( $\beta$ -L-malic acid).

Treatment with aluminum potassium sulfate and tannic acid has been added to support the treatment of anorectal diseases. Functional analysis of the anorectum with defecography

and stationary 3-dimensional manometry have been introduced to help us to better understand anorectal function and to improve treatment strategies in patients with anorectal functional disorders.

### *Hepatobiliary and pancreatic surgery*

The outlines of our main research activities are as follows.

1. Living donor liver transplantation (LDLT) and regenerative medicine
2. Treatment of hepatocellular carcinoma (HCC) and control of recurrence
3. Chemotherapy for pancreatic and biliary cancers
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 surgery
7. Nutritional therapy for patients with cancer
8. Surgical site infection control
9. Effect of preoperative treatment with eltrombopag on splenectomy for idiopathic thrombocytopenic purpura
10. Genome-wide association study of donors and recipients in LDLT
11. Molecularly targeted therapy for advanced HCC
12. Analyses of new biological tumor markers for HCC

The first LDLT was successfully performed for a patient with postnecrotic cirrhosis and HCC on February 9, 2007. Our 10th LDLT was performed on March 16, 2012, for a patient with recurrent primary sclerosing cholangitis who had previously undergone LDLT for primary sclerosing cholangitis at our hospital in October 2009. All 10 recipients were discharged on postoperative days 15 to 46 in good condition, and all donors were discharged on postoperative days 8 to 13 and have returned to their preoperative status. We are planning to extend the indications of LDLT to ABO-incompatible cases and to acute hepatic failure. We have conducted translational research on combination chemotherapy with gemcitabine and a novel protease inhibitor, FUT-175, which is associated with both nuclear factor  $\kappa$ -B inhibition and apoptosis induction in pancreatic cancer cell lines. Navigation surgery for liver surgery was adopted by the national health insurance system as of April 1, 2012, and biliary and pancreatic navigation surgery is performed with the Institute for High Dimensional Medical Imaging Research Center. Other clinical and experimental trials on the treatment of hepatic tumors, laparoscopic surgery, nutritional therapy, surgical site infections, and eltrombopag as a pretreatment for laparoscopic splenectomy for idiopathic thrombocytopenic purpura, are ongoing. Also, we are participating in multicenter studies of genome-wide association, molecularly targeted therapies for advanced HCC, and new biological tumor markers for HCC.

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## Department of Surgery

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

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#### General Summary

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

#### Research Activities

##### *Chest Surgery*

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

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

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

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

Our clinical studies are also evaluating new devices and methods, such as narrow-band imaging for the thoroscopic diagnosis of benign and malignant lung diseases, and Laparoscopic coagulating shears (Ethicon Endo-Surgery, Inc, Cincinnati, OH, USA) for small thoracotomy. Three-dimensional diagnosis with computed tomography is used to make thoracoscopic surgery safer. The diagnosis and treatment of ground glass opacity of the lung, which is considered to indicate early adenocarcinoma, are being evaluated.

Many basic research studies are also underway. In the morphological expression-related advancement of the molecular genetic analysis of lung cancer, we are investigating whether carcinogenesis of the lung, as reflected by CA19-9 activity is an important marker of de novo carcinogenesis. The biological and genetic characteristics of peripheral adenocarcinoma of the lung are being investigated to establish the most appropriate surgical procedures.

A system for viewing videos on the Internet is now being developed to improve surgical training and research.

### *Breast and Endocrinology Surgery*

With the spread of screening mammography in Japan, ductal carcinoma in-situ now accounts for 20% of all breast cancers. We have studied biological factors involved in the progression of ductal carcinoma in-situ to invasive breast cancer by immune-staining procedures.

We have performed phase II/III studies of contrast-enhanced ultrasonography of the breast with Sonazoid (Daiichi Pharmaceutical Co., Ltd., Tokyo) in cooperation with the Department of Radiology. For small cancers of the breast, Sonazoid increases the sensitivity of ultrasonography to equal that of magnetic resonance.

Triple-negative breast cancer (TNBC) is often associated with early resistance to chemotherapy and extremely poor outcomes. Neoadjuvant chemotherapies have demonstrated efficacy in some patients with TNBC. By analyzing clinicopathological data, we have identified chemosensitivity factors in TNBC.

Sentinel lymph-node navigation has become a standard procedure in breast cancer surgery worldwide. However, the use of sentinel lymph-node biopsy after preoperative chemotherapy remains controversial. We are investigating its validity for standard use, especially after preoperative chemotherapy.

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 circulating tumor cells in the bone marrow for survival in patients receiving chemotherapy.

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## Department of Surgery

### Division of Pediatric Surgery and Vascular Surgery

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Takao Ohki, *Professor and Chairperson*  
 Yuji Kanaoka, *Assistant Professor*  
 Joji Yoshizawa, *Assistant Professor*

Atsushi Ishida, *Assistant Professor*  
 Naoki Toya, *Assistant Professor*

#### General Summary

##### *Pediatric Surgery*

The Division of Pediatric Surgery at The Jikei University Hospital is dedicated to providing expert surgical care for fetuses, infants, children, and adolescents with congenital and acquired conditions. Our surgeons remain committed to the ongoing development of new surgical techniques for treating diseases in children—particularly minimally invasive approaches to replace more invasive open procedures that require large incisions.

##### *Vascular Surgery*

Research projects of our department have focused on the development of endovascular repair of aneurysms, treatment of peripheral arterial disease with drug-eluting stents, and clinical study of specific antibodies for heparin-platelet factor 4 (PF4) complexes.

#### Research Activities

##### *Pediatric Surgery*

##### 1. Education

Education for medical students: Many children undergoing surgery have congenital anomalies. Therefore, lectures in pediatric surgery for students are based on embryology. Three students have attended overseas lectures at the Department of Pediatric Surgery of Stanford University.

Education for training doctors: Three objectives for training doctor in pediatric surgery are: 1) how to obtain a blood sample from pediatric patients, 2) understanding fluid therapy for pediatric patients, and 3) learning how to create buried sutures.

Education for surgical residents: Residents are able to perform as operators or assistants for pediatric surgery

##### 2. Clinical studies

##### a. Endoscopic treatment for vesicoureteral reflux using Deflux®

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

##### b. Electrolytes and acid-base balance in laparoscopic surgery

Carbon oxide produces changes in electrolytes and the acid-base balance in laparoscopic surgery.

##### c. In severe cases of gastroesophageal reflux, a surgical procedure called fundoplication

is performed. This procedure is performed laparoscopically in our hospital. With minimally invasive laparoscopic surgery, pain is minimized, and the postoperative recovery time is shorter. The number of neurologically handicapped children treated at our hospital for gastroesophageal reflux has been increasing.

d. The Nuss procedure for treating pectus excavatum aims to force the sternum forward and hold it there 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 research

#### a. Laparoscopic surgery contributes to global warming

Carbon dioxide, the most important greenhouse gas, is indispensable for laparoscopic surgery. To assess CO<sub>2</sub> emissions, we first determined the number of laparoscopic operations performed in Japan. Next, we measured the quantity of CO<sub>2</sub> used in our hospital.

#### b. Inhibitory effects of antiangiogenesis drugs on the metastasis of human neuroblastoma

The loss of antiangiogenesis factors was discovered. We evaluated the effects of several potent antiangiogenesis drugs on the metastasis of neuroblastoma in a mouse model of liver metastasis.

#### c. Plasmapheresis in severe sepsis or septic shock

During sepsis, microorganisms release various endotoxins that activate cascade systems, including cytokines, such as tumor necrosis factor alpha and interleukin 6, and complement components. Plasmapheresis is used to remove these factors. We created a rat model of sepsis and evaluated the effects of plasmapheresis.

### *Vascular Surgery*

#### 1. Development of endovascular repair of thoracoabdominal aneurysm

Although stent graft for the treatment of abdominal aortic aneurysm (AAA) has been established and is commercially available, no such stent grafts are available for the treatment of thoracoabdominal aortic aneurysm (TAAA). Although the surgical death rate following open surgery for the treatment of AAA is satisfactory, that for TAAA remains unacceptably high at 15% to 20%, and further improvement is desperately needed. Because TAAA involves one or more visceral arteries, visceral perfusion must be maintained while excluding the aneurysm with stent graft. We have used a custom-made branched stent graft in combination with covered stents (for visceral reconstruction) for the treatment of TAAA that was considered inoperable because of co-morbid conditions or a hostile thorax/abdomen. Although stent graft repair for TAAA requires long operative and fluoroscopic times, this treatment is feasible and safe.

#### 2. Development of endovascular repair of aortic arch aneurysm: Retrograde in-situ branched surgery

We have developed a new minimally invasive operation for aortic arch aneurysm. After carotid-carotid bypass surgery is performed and stent graft are placed, a needle is used to push the stent graft through one side of a carotid artery, after which a covered stent is inserted as a branch and deployed into the stent graft (in an *in-situ* retrograde fashion).

ion). 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 aneurysm.

### 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 for treating peripheral arterial disease affecting the superficial femoral artery, the main vessel of the thigh. The Zilver PTX is a self-expanding stent made of nitinol, a space-age “shape memory” metal that offers unique mechanical advantages for a stent in the superficial femoral artery.

We participated in a global registry and randomized controlled trial with patients from United States, Germany, and Japan which reached its 1-year primary endpoint in August 2009. With this trial, the Zilver PTX received approval from the Japanese Pharmaceuticals and Medical Devices Agency in January 2012 and will soon be 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 the symptoms of HIT, particularly in patients undergoing major vascular surgery. We measured these variables in 300 patients for 2 years.

The percentage of patients with antibodies against 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 in 2012.

### 5. Research on prevention of reperfusion injury during endovascular aneurysmal repair

Large sheaths are usually chosen 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 used a small sheath 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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Mitsuru Saito, *Associate Professor*  
Mamoru Yoshida, *Assistant Professor*  
Hideki Fujii, *Assistant Professor*

Kazuo Asanuma, *Associate Professor*  
Takuya Otani, *Associate Professor*  
Shigeru Soshi, *Associate Professor*  
Makoto Kubota, *Assistant Professor*  
Yutaka Ueno, *Assistant Professor*

### General Summary

#### *Basic Research*

Our research, ranging from connective tissue cells to clinical research, has been widely acknowledged both from within Japan and overseas. We have identified a risk factor for severe vertebral compression fractures and are proposing tailor-made therapies to improve both bone density and bone quality.

#### *Clinical Research*

There is a variety of specialties within orthopaedics, but since the appointment of Professor Marumo our department has created a clinical environment for treating all motor disorders. Clinics have been separated into various specialties, including the knee, hip, spine, shoulder, hand, foot, trauma, bone metabolism, and rheumatoid disease, to meet patient needs. The diverse clinical treatment environment, without the tendency to focus on one specialty, is our philosophy that will continue into the future. Our clinical research, such as the patient-specific templating technique for knee replacement and navigation surgery with real-time computed tomography (CT), is directly connected to the improvement of surgical techniques. The development of surgical techniques is important for surgical outcomes and is an essential aspect of research at this university hospital.

### Research Activities

#### *Comparison of magnetic resonance findings before and after nonsurgical treatment in patients with full-thickness tears of the rotator cuff*

The purpose of this study was to compare magnetic resonance (MR) findings before and after nonsurgical treatment in 18 patients with full-thickness tears of the rotator cuff. The relationships between improvements in MR findings, age, tear size, interval between follow-up MR studies, pain score, and range of motion were evaluated retrospectively. Thirteen patients had no changes in MR findings. The other 5 patients showed improvements of the subacromial bursa, glenohumeral joint, and the sheath of the tendon of the long head of the biceps. The improvements in symptoms were not directly associated with the MR findings. The improvement in MR findings was suspected to appear more than 6 months after the improvement in symptoms.

*Usefulness of navigation-assisted surgery for spinal deformities using intraoperative 3-dimensional CT images*

Navigation-assisted surgery for spinal deformities was performed with single-time multi-level registration using intraoperative 3-dimensional CT images (Artis zeego, Siemens AG, Erlangen, Germany). The clinical results and the accuracy of navigation were satisfactory.

*The correlation between curve progression and peak height velocity in adolescent idiopathic scoliosis*

The peak height velocity and growth period values in adolescent girls with idiopathic scoliosis were higher and shorter, respectively, than those in healthy girls. The patterns of height velocity curves in girls with idiopathic scoliosis differed from those in healthy girls, suggesting that curve progression is associated with the magnitude of the peak height velocity and the length of the growth period.

*Clinical outcomes of multilevel spinal fixation with the minimally invasive spine stabilization technique*

We investigated clinical outcomes of multilevel fixation (3 or more levels) in patients who were treated with minimally invasive spine stabilization. The results were satisfactory with no complications. Therefore, the minimally invasive spine stabilization procedure for multilevel fixation is effective for decreasing invasiveness.

*Patient-specific templating method in total knee arthroplasty: A prospective study of 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-specific bone-cutting guides 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 the development of more precise preoperative planning software. The comparative analysis of the accuracy of different patient-specific cutting guides is carried out in a prospective manner.

*A rotation-free modular femoral stem in primary total hip arthroplasty for hip dysplasia*

Short-term clinical results were evaluated for 219 primary total hip arthroplasty procedures performed with a rotation-free modular femoral stem on secondary osteoarthritis resulting from developmental dysplasia of the hip. The results showed that this modular system has the morphological advantage of an infinite ability to correct rotational deformity of the proximal femur, which is frequently seen in patients with developmental dysplasia of the hip. Rotational alignment of the stem neck was corrected so that the stem anteversion was decreased in 56% of patients by up to 60° and was increased in 18% of patients by up to 45°. The results also revealed functional advantages, with an extremely high probability (99.5%) of obtaining secure bone ingrown fixation of the stem on radio-

graphs and an extremely low incidence (0.9%) of postoperative dislocation.

*Review of symptomatic accessory navicular in patients with acquired progressive flatfoot*

We reviewed symptomatic accessory navicular in patients with acquired progressive flatfoot. The patients had an accessory navicular, but there was no degeneration of the tibialis posterior tendon. We performed excision of the the accessory navicular, reattachment of the tibialis posterior tendon, and correction osteotomy. The flat feet were corrected, and the pain was reduced. Recent studies have shown that the presence of an accessory navicular causes rupture of the plantar calcaneonavicular ligament and the progression of flatfoot. We believe that the disruption of the connection between the navicular and the accessory navicular leads to ineffective force transmission of the tibialis posterior, which results in the progression of flatfoot. We suggest that in such cases, flatfoot must be treated and the symptomatic accessory navicular should be resected.

*A new system for evaluating beta-tricalcium phosphate resorbability in opening wedge high tibial osteotomy*

The purpose of this study was to assess bone formation and  $\beta$ -tricalcium phosphate (TCP) resorption after implantation of  $\beta$ -TCP blocks with 60% and 75% porosity in opening high tibial osteotomy (HTO). Several studies have evaluated TCP resorption in opening HTO. However, the results were analyzed only with X-rays. We have recently developed a new evaluation system. We measured CT values of 60% and 75% porosity TCP blocks in the center of CT images at 2 weeks and 6 years. The CT images at 6 years showed that in all cases most of the  $\beta$ -TCP with 75% porosity had been resorbed but that  $\beta$ -TCP with 60% porosity had not been uniformly resorbed. This result indicates that measurement of only certain regions of interest of TCP is inadequate for evaluating TCP resorption. The open-source imaging software program, OsiriX, enabled the whole area to be scanned to measure CT values. This system is useful for evaluating  $\beta$ -TCP resorption and bone formation in any  $\beta$ -TCP-implanted area.

*Plasma homocysteine levels associated with severity of vertebral fracture in postmenopausal women*

**Purpose:** The aim of this cross-sectional study was to clarify additional risk factors for severe vertebral fractures in postmenopausal Japanese women.

**Methods:** At the registration of this cohort, age, body-mass index, bone mineral density (BMD), and present illness were investigated. Biochemical variables, including urinary levels of type I collagen cross-linked N-telopeptides (NTX) and pentosidine and plasma levels of homocysteine, were measured. Measurements were compared with different vertebral fracture grades (grade 0 to 4). Independent risk factors for severity of vertebral fracture were evaluated by multiple logistic analysis.

**Results:** A total of 1,475 participants ( $66.6 \pm 9.0$  years) were included. Distribution of vertebral fracture grades was grade 0, 1,052 cases (71.3%); grade 1, 137 cases (9.3%); grade 2, 124 cases (8.4%); and grade 3, 162 cases (11.0%). Age, lumbar BMD, urinary NTX, urinary pentosidine, serum homocysteine, and presence of hypertension were significantly increased in accordance with vertebral fracture severity. When comparing

vertebral fracture grade 0 versus grade 2 to 3, pentosidine and homocysteine levels were significant risk factors for vertebral fracture severity (odds ratio [OR]: 1.03, 95% confidence interval [CI]: 1.00–1.06,  $P=0.042$ ; OR: 1.06, 95% CI: 1.00–1.06,  $P=0.013$ , respectively). Homocysteine levels were also a significant risk factor when comparing vertebral fracture grade 0 versus grade 3 (OR: 1.08, 95% CI: 1.02–1.15,  $P=0.006$ ).

**Conclusion:** Levels of pentosidine and homocysteine are independently associated with the severity of vertebral fracture. In particular, homocysteine levels play an important role in the severity of vertebral fracture.

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## Department of Neurosurgery

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 Satoshi Ikeuchi, *Associate Professor*  
 Yuzuru Hasegawa, *Associate Professor*  
 Tatsuhiro Joki, *Assistant Professor*  
 Toshihide Tanaka, *Assistant Professor*

### General Summary

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

### Research Activities

#### *Cerebrovascular disorders*

Although cerebral vasospasm is a major cause of morbidity and mortality in patients with subarachnoid hemorrhage (SAH), precise mechanisms responsible for the pathogenesis of cerebral vasospasm remain undefined. Recent electrophysiologic and pharmacological studies show that potassium channels play important roles in the hyperpolarization and relaxation of vascular smooth muscle. Therefore, we have attempted to determine the role of potassium channels in the relaxation of cerebral arteries and arterioles. The recent results suggest that the functions of potassium channels are potentiated in arteries exposed to SAH and that the role of potassium channels may be more important in small arterioles than in large cerebral arteries.

In thrombolytic therapy for acute ischemic stroke, it is essential to achieve thrombolysis before ischemic neuronal injury occurs. To develop a new technique of thrombolysis after acute stroke, the effect of transcranially applied ultrasound on thrombolysis has been examined. We have reported that low-frequency and low-intensity transcranially applied ultrasound can enhance thrombolysis by tissue plasminogen activator in a rabbit model of femoral artery occlusion. Furthermore, our recent results show that ischemic neurological deficits can be reduced by transcranially applied ultrasound in a rabbit model of middle cerebral artery occlusion without an increase in the rate of hemorrhagic complications. We have reported these results in an international journal (*Stroke*). We are now attempting to confirm the safety of ultrasonication for vascular and neuronal tissue and to develop a clinically applied ultrasonication probe.

#### *Endovascular surgery*

1. Development of a new endovascular operating system

We performed several clinical and basic research studies related to endovascular therapy.

## 2. Development of a new endovascular operating system

We developed a state-of-the-art endovascular neurosurgery suite that offers integrated neurosurgical and radiological capabilities. A specially designed biplane digital subtraction angiography system was installed in the neurosurgery operating room. In May 2008, a robotic digital subtraction angiography system (Zeego, Siemens Medical Systems, Erlangen, Germany) was installed in our operating suite. The new suite, which has 3-dimensional digital subtraction angiography imaging and microsurgery capabilities, allows neurosurgeons to perform a wide array of neurosurgical and endovascular procedures.

## 3. Development of bioactive coils (Matrix coil)

We developed a biodegradable, bioabsorbable polymer coil for the treatment of brain aneurysms at University of California Los Angeles (UCLA) School of Medicine. This device has been approved and has been used to treat more than 30,000 patients in the United States, Europe, and Japan. We are collaborating with UCLA, and the next generation of bioactive coil is being investigated at the Jikei Animal Laboratory. We are planning new clinical research for the treatment of unruptured intracranial aneurysms.

## 4. Development of Mebiol gel

We have developed a thermoreversible polymer as a tissue-engineering therapeutic device. This polymer can be used as a drug delivery embolic material for the treatment of malignant tumors or as a hemostatic device.

We obtained a grant for this project from the New Energy and Industrial Technology Development Organization. We have used this device to treat cerebral aneurysms, and preliminary data hold promise for clinical application.

## 5. Flow dynamics for intracerebral aneurysm

The aim of this project was to predict the risk of rupture of untreated cerebral aneurysms and to develop next-generation therapies that can be used to modify the flow dynamics of the aneurysms. In collaboration with Waseda University, we established a new variable, "energy loss," which can be used to predict aneurysm rupture. In addition we developed a new computational software program that can be used to measure aneurysm size and volume immediately using 3-dimensional information. This software will be commercially available soon.

### *Brain tumor*

In the treatment of malignant glioma, local recurrence often determines prognosis. The principal of therapy thus becomes the control of local recurrence. However, treating local recurrence with chemotherapy is difficult because the blood-brain barrier is a major obstacle preventing chemotherapeutic drugs from reaching brain tumors. To overcome these problems, a method has been developed for the local sustained release of chemotherapeutic agents by their incorporation into biodegradable polymers. Gliadel Wafer (Eisai Co., Ltd., Tokyo, Japan), which contains carmustine, has been authorized in Europe and the United States and is used for the patients with malignant glioma. On the other hand, recent advances in liposome technology have shown promise for the introduction of chemotherapeutic agents with reduced toxicity, extended longevity, and potential for cell-

specific targeting. In some previous reports, liposomal doxorubicine was used systemically to treat malignant glioma. In our study we have tried to use doxorubicine and a proteasome inhibitor (MG132) within a thermoreversible polymer for intracranial implantation, a strategy that has been shown to be safe and successful in the treatment of malignant gliomas. We will investigate the release kinetics, toxicity, distribution, and efficacy of this preparation in vitro and in vivo.

We investigated the safety and clinically effects of immunotherapy with fusions of dendritic and glioma cells in patients with malignant glioma. Dendritic cells were generated from the peripheral blood. Cultured autologous glioma cells were obtained from surgical specimens in each case. Fusions of dendritic cells and glioma cells were prepared with polyethylene glycol. All patients received 3 to 7 immunizations with fusion cells at intervals of 3 weeks. Fusion cells were injected subcutaneously close to a cervical lymph node. There were no serious adverse effects, and partial responses have been observed in 2 patients.

#### *Neurotrauma*

Few institutions have engaged in research on neurotraumatology. A unique aspect of our department is research in this area, which has 3 major topics. 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 also examined sports-related concussion and performed mechanical studies of head-injury through simulations.

#### *Syringomyelia*

About 50 patients with syringomyelia are surgically treated in our department each year. We have been investigating the following subjects.

1. Evaluation of the cerebrospinal fluid obstruction at the craniovertebral junction in patients with Chiari malformation

In syringomyelia related to Chiari malformation, the relation between cerebrospinal fluid (CSF) circulation blockage and cavitation of the spinal cord has been clarified. Therefore, the improvement of 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., the dens) compress the spinal cord and restrict CSF circulation. We examined whether these 2 factors influence the effects of foramen magnum decompression.

2. Fluid in the syrinx

The mechanism of syrinx enlargement remains unclear. The content of the syrinx is believed to be CSF, but where and how the fluid originates are unknown. We are researching the fluid by measuring cytokine and antibiotic concentrations.

#### *Spine and spinal cord group*

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

interests of patient-orientated 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.

### *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 more than 1,500 new cases of various entities have been collected and recorded in our data bank, including, spina bifida, hydrocephalus, craniofacial anomalies, and brain tumors. Since April 2003, clinical research fellows, 12 from other domestic universities and 9 from other countries (including Germany, Italy, Austria, Jordan, and Bulgaria), have taken part in our research activities.

In the field of hydrocephalus research, pathophysiological analyses of CSF dynamics in both the fetal and postnatal periods have been extensively investigated. On the basis of these large clinical series with extensive clinical investigations, we have proposed a unique theory for the specificity of CSF dynamics in the immature brain, namely "Evolution Theory in CSF Dynamics" (Childs Nerv Syst 22: 2006).

We have also completed the development of a new neuroendoscope and proposed a new surgical technique (*J Neurosurg*: 102, 2005) and a specific technique for intracranial cysts (*J Neurosurg*: 103, 2005) with a specific navigational endoscope trajectory as "Oi clear Navi Sheath" (*J Neurosurg*: 107, 2007). We have been collecting the largest series of patients.

A member of our department has been nominated as the chairman of the National Study Group on Spina Bifida and has been promoting further nationwide 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 has been summarized, and our extensive work 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 been well maintained both in Tokyo (The Jikei University Hospital Women's & Children's Medical Center) and in Hannover, Germany (the International Neuroscience Institute) on the basis of firm international collaboration with world-leading pediatric neurosurgeons and related research workers.

### **Publications**

**Murayama Y, Irie K, Saguchi T, Ishibashi T, Ebara M, Nagashima H, Isoshima A, Arakawa H, Takao H, Ohashi H, Joki T, Kato M, Tani S, Ikeuchi S, Abe T.** Robotic digital subtraction angiography systems within the hybrid operating

room. *Neurosurgery*. 2011; **68**: 1427-32.

**Tani S, Nagashima H, Isoshima A, Akiyama M, Ohashi H, Tochigi S, Abe T.** A unique device, the disc space-fitted distraction device for anterior cervical discectomy and fusion: early clinical and

radiological evaluation. *J Neurosurg Spine*. 2010; **12**: 342-6.

**Kato N, Tanaka T, Sakamoto H, Arai T, Hasegawa Y, Abe T.** Identification of a persistent primitive trigeminal artery following the transposition technique for trigeminal neuralgia: a case report. *Pain Res Manag*. 2011; **16**: 357-9.

**Sakamoto H, Tanaka H, Kato N, Arai T, Hasegawa Y, Abe T.** Frontal sinus mucocoele with intracranial extension associated with osteoma in the anterior cranial fossa. *Neurol Med Chir (Tokyo)*. 2011; **51**: 600-3.

## Reviews and Books

**Tanaka T, Kato N, Arai T, Hasegawa Y, Abe T.**

Hemangiopericytoma in the trigone or the lateral ventricle. *Neurol Med Chir (Tokyo)*. 2011; **51**: 378-82.

**Terao T, Ishii T, Tani S, Abe T.** Combination therapy of radiofrequency lumbar facet joint denervation and epidural spinal cord stimulation for failed back surgery syndrome. *Neurol Med Chir (Tokyo)*. 2011; **51**: 805-9.

**Arai T, Horiguchi K, Saeki N, Oka H, Saito T, Takahashi-Fujigasaki J, Sakamoto H, Kato N, Dobashi H, Tanaka T, Hasegawa Y, Abe T.** Surgical treatment of a calcified Rathke's cleft cyst with endoscopic extended transsphenoidal surgery. *Neurol Med Chir (Tokyo)*. 2011; **51**: 535-8.

## Department of Plastic and Reconstructive Surgery

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Mitsuru Uchida, *Professor*  
 Kunitoshi Ninomiya, *Associate Professor*  
 Kimihiro Nojima, *Assistant Professor*

Takeshi Miyawaki, *Associate Professor*  
 Shintaro Matsuura, *Assistant Professor*  
 Yoko Kishi, *Assistant Professor*

### General Summary

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

### Research Activities

#### *Gene analysis and staged surgical procedures in patients with syndromic craniosynostosis*

Apert syndrome, or acrocephalosyndactyly I, is an autosomal dominant disease caused by allelic mutations of fibroblast growth factor receptor 2 (FGFR2). Two regions (Ser 252 Trp and Pro 253 Arg) of the FGFR2 gene are believed to be responsible for syndromic craniosynostosis. Four monoclonal antibodies that respond only to peptides derived from mice with a mutation of Pro 253 Arg have been successfully prepared.

#### *Treatment after total necrosis of free flap reconstruction for head and neck cancer*

Of more than 300 patients who underwent free flap reconstruction after excision of head and neck cancers from January 2005 through December 2009, less than 5% of the patients had total flap necrosis. The flaps with total necrosis included 7 free jejunum flaps, 3 rectus abdominis musculocutaneous flaps, 2 anterolateral thigh flaps, and 2 fibular bone flaps. As soon as the necrosis of free jejunum flaps was recognized, salvage procedures were performed. Free flap reconstruction was performed in 6 patients, and the graft survived in 5 patients. Considering functional and cosmetic aspects, free flap retransplantation is desirable as a salvage strategy for total flap necrosis. When free flap reconstruction is difficult, a pedicled flap may be an option if some degree of function and cosmetic effects can be maintained.

#### *Evaluation of flap vascularization with intraoperative and postoperative infrared thermal imaging*

Success rates of free flap reconstruction of large defects due to excision of malignant

tumors are high (at least 95%). Wound dehiscence and other complications, however, are occasionally seen, especially in patients who have received radiation or chemotherapy or both. Infrared thermography (TVS-200EX, NEC Avio Infrared Technologies, Ltd., Tokyo) is a reliable, noninvasive technique for assessing the vascularization and viability of free flaps and surrounding tissue. It is a useful method for monitoring free flaps and provides valuable information for avoiding complications.

#### *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 for bone distraction and temporary traction of joints. Use of the Ilizarov minifixator is an effective and noninvasive method and is highly recommended in selected cases.

#### *Distraction osteogenesis*

The use of distraction osteogenesis in reconstruction continues to expand and evolve. The effects of the various rates and frequencies of distraction have been studied, and a rate of 1 to 2 mm per day has been found to be adequate for the craniofacial skeleton. The division of daily distractions into smaller, more frequent distractions accelerates bone formation. We have developed a device with a built-in motor which can produce continuous distraction. Results of experiments using newly developed devices are being investigated.

#### *Tissue engineering*

Flaps lined with mucosa are in great demand for nasal, oral, tracheal, and urogenital reconstruction. Fascia lined by mucosal tissue has already been developed as a new reconstructive material. Sublingual mucosa was obtained from Japanese white rabbits, and separated mucosal cells were subcultured twice for 4 weeks. The cells were transplanted to the fascia of the femoral muscles in the same rabbits. Histological examination confirmed the growth of mucosal tissue. Fasciomucosal complex tissue developed. Fascia proved to be a useful scaffold that cross-links the transplanted mucosa and muscle.

#### *Functional analysis of desert hedgehog in patients with macrodactyly*

Mou reported in 2008 that the expression of the protein desert hedgehog in the hypertrophic parts of affected nerves was significantly greater in patients with macrodactyly than in patients with polydactyly. The purpose of the study was to detect expression of the messenger (m) RNA of desert hedgehog and immunohistochemical reactions for desert hedgehog and Patched2 in the fatty tissues of patients with macrodactyly. Immunohistochemical reactions for desert hedgehog were observed in the epidermis and adipocytes of patients with macrodactyly, whereas mRNA reactions were detected in the nervous systems of both patients with macrodactyly and patients with polydactyly. Whether the up-regulation of desert hedgehog is due to the disease itself or is a consequence of surgery is

unclear, and further investigation is planned.

## Publications

**Matsuura S, Uchida M.** Use of Flaps in reconstruction of the hand (in Japanese). *Keiseigeka*. 2011; **54**: 765-74.

**Sakai S, Uchida M, Takeishi M, Miyawaki T.** Algorithm in the treatment of synbrachydactyly (in Japanese). *Nihon Tegeka Gakkai Zasshi*. 2010; **27**: 307-11.

**Matsuura S, Uchida M.** Extra-fixation for fracture of distal phalanx (in Japanese). *Seikeigeka Saisho Sinshu Shujutsu Janaru*. 2011; **61**: 11-5.

**Matsuura S, Miyawaki T, Soga M, Hayashi J,**

**Uchida M, Ishibashi K.** Usefulness of Ilizarov mini fixator for hand surgery (in Japanese). *Nihon Sogai Kotei Kotsuencho Gakkai Zasshi*. 2012; **23**: 15-22.

**Ninomiya K, Nakahara M, Paku S, Yokawa Y, Hirakawa M, Nishimura R.** Clinical study on the closed rupture of the extensor pollicis longus tendon following fractures of the distal end of the radius (in Japanese). *Keiseigeka*. 2012; **55**: 301-7.

## Department of Cardiovascular Surgery

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Kiyozo Morita, *Professor*  
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 Yoshimasa Uno, *Assistant Professor*  
 Hirokuni Naganuma, *Assistant Professor*

### General Summary

The main investigation in our department involved clinical study, evaluation of alterations in cardiac performance and long-term results after corrective surgery, and experimental studies to solve the clinical problems we are facing. Clinical investigations, including follow-up studies, of valvular and ischemic heart diseases were a main area of our clinical research, as were studies of complex congenital anomalies. Recent topic for adult surgery is mitral valve plasty in the cases of acute infective endocarditis. The recent increase in aortic aneurysms has continued and surgical strategy was established. We are also continuously performing several experimental studies with in vivo models. The experimental projects include protection of the heart during cardiac arrest and pulmonary valve function. The major activities are described below.

### Research Activities

*Experimental studies of congenital heart diseases: The effect of cardiopulmonary factors on the severity of pulmonary regurgitation in an acute swine model*

The progression of pulmonary regurgitation after intracardiac repair for congenital heart defects requiring right ventricular (RV) outflow tract reconstruction results in the RV volume overload and subsequent RV dysfunction, contributing to poor morbidity and reoperation. We examined the effects of cardiopulmonary factors (RV systolic function [end-systolic elastance,  $E_{es}$ ] and pulmonary vascular resistance index [PVRI]) on the severity of pulmonary regurgitation (PR) in an acute swine cardiopulmonary bypass (CPB) model. In 8 pigs (body weight,  $14 \pm 2$  kg), an acute PR model was established with the use of CPB. The severity of PR (%PR) assessed with a Doppler flow meter (backward/forward flow area) was  $40\% \pm 4\%$  at the steady state after the operation. During the serial alterations of PVRI by manipulation of ventilation and NO inhalation, %PR increased in parallel with PVRI ( $p < 0.01$  %PR versus PVRI). Furthermore, %PR was reduced by stepwise increases in RV  $E_{es}$  by dobutamine infusion. In conclusion, the hemodynamic effect of PR depends on the patient's cardiopulmonary status (i.e., RV function and pulmonary vasoconstriction) in addition to the status of pulmonary valve competence.

*Experimental studies of new therapeutic strategies of cardiopulmonary protection during open-heart surgery*

1. Reversal of oxidant-mediated biochemical injury and prompt functional recovery after prolonged single-dose crystalloid cardioplegic arrest in immature piglet heart by the

terminal warm-blood cardioplegia supplemented with a phosphodiesterase III inhibitor Terminal blood cardioplegia (TWBCP) alone provides insufficient benefits after prolonged ischemia and is associated with inevitable oxidant-mediated injury. To examine methods of avoiding oxidant-mediated myocardial reperfusion injury and of facilitating prompt functional recovery, we examined the effects of TWBCP supplemented with high-dose olprinone, a phosphodiesterase III inhibitor, which has the potential to reduce oxidant stress and calcium overload, after prolonged single-dose crystalloid cardioplegic arrest in a model of CPB in immature piglets. Fifteen piglets were subjected to 90 minutes of cardioplegic arrest on CPB, followed by 30 minutes of reperfusion. In group I, uncontrolled reperfusion was applied without receiving TWBCP; in group II, TWBCP was given; and in group III, TWBCP was supplemented with olprinone (3  $\mu\text{g}/\text{ml}$ ). Group III showed significant left ventricular (LV) performance recovery (Group I,  $26.5\% \pm 5.1\%$ ; group II,  $42.9\% \pm 10.8\%$ ; group III,  $81.9\% \pm 24.5\%$ ,  $p < 0.01$  versus groups I and II), associated with significant reduction of troponin T and lipid peroxidation at the reperfusion phase. In groups III no piglets required electrical cardioversion. On the basis of this study, we conclude that TWBCP with olprinone reduces myocardial reperfusion injury by reducing oxidant-mediated lipid peroxidation and accelerates prompt and persistent LV functional recovery while suppressing reperfusion arrhythmia.

2. Effect of postconditioning: Experimental study using a piglet model of cardiovascular surgery on the reversal of myocardial stunning by ischemic postconditioning

Background: This study tested the hypothesis that myocardial damage induced by ischemia/reperfusion can be reduced by postconditioning at reperfusion.

Methods: Eighteen piglets were subjected to 90 minutes of ischemia followed by 60 minutes of reperfusion on CPB. In 12 of them, ischemic postconditioning strategies (6 cycles of 10 seconds of ischemia/reperfusion or 3 cycles of 30 seconds of ischemia/reperfusion) were applied before aortic unclamping, whereas the other 6 were not treated (control).

Results: In the LV, both systolic and diastolic dysfunction, associated with oxidant-induced biochemical injury, were noted in the control group. In contrast, postconditioning resulted in significantly better LV functional recovery and less myocardial biochemical injury.

Conclusion: Ischemic postconditioning during the early phase of reperfusion produces prompt myocardial functional recovery and inhibits biochemical injury in a piglet model of CPB.

### *Clinical studies of pediatric heart surgeries*

1. Postoperative changes in coagulability and fibrinolytic function in Fontan circulation: Possibility of the conversion of anticoagulation therapy

There is still no consensus concerning the postoperative use and duration of treatment with warfarin as an anticoagulant in patients undergoing the Fontan procedure. We evaluated the changes in coagulability and fibrinolytic function after the surgery and try to use them as the indicator of anticoagulation therapy. Plasma levels of thrombin anti-thrombin-3 complex (TAT), as an index of coagulability, and  $\alpha 2$ -plasmin inhibitor-plasmin complex (PIC), as an index of fibrinolytic function, were measured in 16 patients

undergoing the extracardiac Fontan procedure (mean age at operation, 4.2 years). Levels of both TAT and PIC remained higher than normal for 6 months after surgery, even in patients treated with warfarin. However, levels of TAT and PIC gradually decreased and had almost normalized by 12 months. On the basis of these results, we have replaced warfarin with an antiplatelet agent in these cases. Even after this change, plasma levels of TAT and PIC have remained normal, and no patients have shown thromboembolic events on echocardiography. This study suggests that patients undergoing the Fontan procedure should receive anticoagulation therapy with warfarin for the first year after surgery because of their activated status of coagulability. However, warfarin can be replaced with an antiplatelet agent 12 months after surgery for patients with normal levels of TAT and PIC and no major complications.

## 2. Effects of oral pulmonary vasodilators (sildenafil and bosentan) in high-risk candidates for the Fontan procedure after the bidirectional Glenn operation

We have retrospectively analyzed the effects of treatment with oral pulmonary vasodilators (sildenafil and bosentan) on the hemodynamic risk profile (pulmonary arterial pressure [PAP], pulmonary vascular resistance: pulmonary resistance [Rp] and PA index) in 8 high-risk candidates for the Fontan procedure and 10 untreated control patients.

In the treatment group, 8 patients who underwent bidirectional Glenn (BDG) operation, significant reductions in Rp and PAP were noted 6 and 12 months after the operation, whereas no changes were demonstrated in the control group. This study suggests that treatment with oral pulmonary vasodilators (sildenafil and bosentan) can reduce pulmonary risk factors in candidates for the Fontan procedure.

## 3. Intraoperative evaluation of pulmonary flow reserve capacity and a new method to predict post-Fontan hemodynamic status

In 12 patients, in whom the staged Fontan procedure was indicated after the BDG operation, we measured superior vena cava flow, which is equivalent to PA flow in BDG physiology, by means of a transit-flow meter intraoperatively. Measurement of PA flow and pulmonary vascular resistance, incorporated with serial volume loading, allows pulmonary vascular reserve capacity to be assessed in response to an increase in pulmonary flow to simulate Fontan circulation. The pulmonary vascular reserve capacity, assessed by the percent reduction in Rp in response to increased pulmonary flow, was revealed to be a strong indicator of post-Fontan outcome and a final central venous pressure (CVP) with Fontan circulation. In 8 patients who had undergone the Fontan procedure, there was significant relationship between the actual CVP and the CVP predicted by means of intraoperative simulation.

## 4. Surgical outcomes and long-term results of the Ross operation: Effect of autograft dilatation

The surgical outcomes and long-term results of the Ross operation were reviewed in 35 patients who undergone the Ross procedure from 1995 through 2008. Autograft function was assessed with periodic echocardiographic evaluation for up to 14 years after the operation. There were no operative or acute deaths or late reoperation for autograft regurgitation (freedom from reoperation for autograft failure: 87% after 14 years). The durability of the implanted pulmonary autograft valve was excellent, especially in children and in patients with preoperative aortic stenosis.

### *Clinical study of adult cardiac surgery*

#### 1. Valve disease

##### 1) Increase of re-do surgery ~ for safer operations

1)-1. *Risk factor*: Recently, the number of re-do surgeries have increased because of the increased number of elderly patients. Most re-do cases involve several severely diseased valves, and the mean interval after the first operation is  $19.6 \pm 9.5$  years. Many patients have cardiac cachexia because of the chronic right heart failure. Moreover, congestive liver damage and splenic hyperactivity cause platelet deficiencies that affect the amount of blood loss during the operation. Although the number of redo surgeries, the amount of blood loss, operation time, and cardiopulmonary bypass time were not risk factors in the our redo surgeries, but renal and liver dysfunction were risk factors for operative mortality.

1)-2. *Surgical approach*: Before sternotomy, DC pads are placed to treat possible arrhythmias, and the femoral artery and vein are kept for cardiopulmonary bypass. The key to dissecting adhesions is to avoid injuring the heart and vessels. To keep from injuries, sternotomy must be done while the previously used wires are pulled upward. It is important to take care of dissecting the adhesion of prosthetic stents, injuries of the coronary orifice, and the left ventricular posterior wall when the old prostheses in the aortic and mitral positions are removed. After removal of the old prostheses, a new annulus is constructed with a pericardial Xenomedica patch if the annulus is defective. We use On-X or MOSAIC valves in the tricuspid position.

##### 2) Valve operation for patients aged older than 80 years

*Increase of the valve surgeries of the elderly patients*: Reflecting the recent aging society, the number of valve operation in our department has been increased in recent years which is close to 10% of the total valve operations. We have to be more careful to choose the adequate surgical approach, thinking over the patient's complications and QOL before operation. Most of valve operations are associated with aortic valve replacements, using bioprosthetic valves and the results are satisfactory. The operative risk is around 5% and the surgical indication should be decided with the preoperative condition and risk scores.

##### 3) Aortic valve replacement in elderly patients

*Effect of patient-prosthesis mismatch*: The frequency of patient-prosthesis mismatch (PPM) was around 12%, which was higher than expected. PPM did not affect survival, and moderate PPM was tolerable. Our exponential curve was different from the original curve that was constructed from the data of 4 different prostheses and defined the criteria of PPM. The mean pressure gradient of the stented bioprosthetic valve demonstrated a gentler slope and a smaller effective orifice area indexed with body surface area compared with the exponential curve described by Pibarot and Dumesnil<sup>1)</sup>. The practical implications of these findings include the necessity to reconsider the hemodynamic performance of each prosthesis when seeking to define PPM, so as to avoid residual significant transvalvular pressure gradient and higher rates of morbidity and mortality.

A) Hemodynamic and clinical impact of prosthesis-patient mismatch in the aortic valve position and its prevention. Pibarot P, Dumesnil JG, J Am Coll Cardiol 2000; 36:1131-41.

#### 2. Ischemic heart disease

### 1) Perioperative management of recent cardiac surgeries

1)-1. *Perioperative management*: It is important to establish specialized medical teams to improve surgical results in an aging society that includes patients with serious complications, such as diabetes and hemodialysis.

**Infection**: The oral cavity is routinely checked by dentists. Cefazolin sodium is administered every 3 hours intraoperatively and for 4 days after the operation. We meet with the infection control team once a week.

**Diabetes**: Blood glucose levels are controlled with the insulin scale before surgery, and continuous insulin infusion maintains postoperative blood glucose levels at less than 180 mg/dl. We routinely consult with diabetes specialists doctor about the control of postoperative blood glucose.

1)-2. *The intensive care unit system*: In our institution, 8 intensive care unit (ICU) specialists and specialists in nephrology, infection control, and other fields help care for patients after surgery in our 20-bed ICU. The cooperative team has contributed to the shortening of the mean ICU stay even though the number of patients with serious comorbidities has increased.

**Rehabilitation**: In the ICU, a cardiovascular physiotherapist begins postoperative rehabilitation.

### 3. Thoracic aneurysm

#### 1) Preventing cerebrovascular complications in aortic arch replacement

The first choice for an aortic infusion line is an ascending aorta without calcification. If there is atherosclerosis, we select an axillary artery. Moreover, we have cannulated atheromatous branches of the aortic arch which have sufficient backflow, achieved by means of selective cerebral perfusion, followed by initial retrograde cerebral perfusion. To prevent the complications of cerebral infarction and air embolism, retrograde cerebral perfusion is a safe and simple method of brain protection.

### Publications

**Sakamoto Y, Takakura H, Onoguchi K, Hachiya T, Sasaki T, Yoshitake M, Hashimoto K.** Cryosurgical left-sided maze procedure in patients with valvular heart disease: medium-term results. *Ann Thorac Cardiovasc Surg.* 2011; **17**: 148-52.

**Kawahito K, Adachi H.** Hypothermic circulatory arrest for acute traumatic aortic rupture associated with shock. *Gen Thorac Cardiovasc Surg.* 2011; **59**: 472-6.

**Kimura N, Tanaka M, Kawahito K, Itoh S, Okamura H, Yamaguchi A, Ino T, Adachi H.** Early- and long-term outcomes after surgery for acute type a aortic dissection in patients aged 45 years and younger. *Circ J.* 2011; **75**: 2135-43.

**Shinohara G, Morita K, Nagahori R, Koh Y, Kinouchi K, Abe T, Hashimoto K.** Ischemic postconditioning promotes left ventricular functional recovery after cardioplegic arrest in an vivo piglet model of global ischemia reperfusion injury

on cardiopulmonary bypass. *J Thorac Cardiovasc Surg.* 2011; **142**: 926-32.

**Nakamura K, Kawahito K.** Time-related hemolysis in stored shed mediastinal blood after cardiopulmonary bypass. *J Artif Organs.* 2011; **14**: 264-7.

**Kawahito K, Naganuma H, Matsumura Y, Nakamura K, Haijima N.** Re-do replacement surgery for degenerated stentless bioprostheses: the open "valve-in valve" technique. *Jikeikai Med J.* 2011; **58**: 117-20.

**Kinouchi K, Morita K, Ko Y, Nagahori R, Shinohara G, Abe T, Hashimoto K.** Reversal of oxidant-mediated biochemical injury and prompt functional recovery after prolonged single-dose crystalloid cardioplegic arrest in the infantile piglet heart by terminal warm-blood cardioplegia supplemented with phosphodiesterase III inhibitor. *Gen Thorac Cardiovasc Surg.* 2012; **60**: 73-81.

**Yamashiro M, Morita K, Uno Y, Ko Y, Mura-**

**matsu K, Hashimoto K.** Malformation of the atrial septum with hemodynamic equivalence to partial anomalous pulmonary venous connection and difficult differentiation from cor triatriatum in an infant (in Japanese). *Nihon Shoni Junkanki Gakkai Zasshi*. 2012; **28**: 48-53.

**Nakamura K, Kawahito K, Matsumura Y, Kagawa H, Hashimoto K.** Severe intestinal ischemia due to chronic aortic dissection: report of a case (in Japanese). *Kyobugeka*. 2012; **65**: 161-4.

## Department of Obstetrics and Gynecology

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 Satoshi Takakura, *Assistant Professor*  
 Hiroshi Tanabe, *Assistant Professor*  
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Kazunori Ochiai, *Professor*  
 Hiroshi Sasaki, *Professor*  
 Naoki Kamiya, *Professor*  
 Shigeki Niimi, *Associate Professor*  
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 Seiji Wada, *Assistant Professor*

### General Summary

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

### Research Activities

#### *Gynecologic oncology*

1. High-risk ovarian cancer based on a 126-gene expression signature is uniquely characterized by downregulation of the antigen-presentation pathway

High-grade serous ovarian cancers are heterogeneous both in terms of clinical outcomes and at the molecular level. Our aim was to establish a novel risk-classification system based on gene expression signatures for predicting overall survival which we hope will lead to novel therapeutic strategies for high-risk patients. In this large-scale cross-platform study of 6 microarray data sets from 1,054 patients with ovarian cancer, we developed a gene expression signature for predicting overall survival by applying elastic net analysis and 10-fold cross-validation to Japanese data set A (n=260) and evaluated signatures in 5 other data sets. Subsequently, we investigated differences in biological characteristics between patients with high-risk and low-risk ovarian cancers. Elastic net analysis of Japanese data set A identified a 126-gene expression signature for predicting overall survival in patients with ovarian cancer (multivariate analysis,  $P=4 \times 10^{-20}$ ). We validated its predictive ability through multivariate analysis with 5 other data sets (Tothill's data set,  $P=1 \times 10^{-5}$ ; Bonome's data set,  $P=0.0033$ ; Dressman's data set,  $P=0.0016$ ; The Cancer Genome Atlas data set,  $P=0.0027$ ; Japanese data set B,  $P=0.021$ ). Through gene ontology and pathway analyses, we identified a significant reduction in expression of immune-response-related genes, especially on the antigen-presentation pathway, in patients with high-risk ovarian cancer. This risk classification based on a 126-gene expression signature is an accurate predictor of clinical outcome in patients with advanced high-grade serous ovarian cancer and might lead to new therapeutic strategies for patients with high-grade serous ovarian cancer.

2. Short-term serum deprivation confers sensitivity to taxanes in platinum-resistant

human ovarian cancer cells

On the basis of evidence that serum deprivation provokes apoptosis in variety of cells, the effect of serum deprivation on drug sensitivity was examined. Serum deprivation resulted in significant increases in paclitaxel sensitivity by factors of 148 and 10 in platinum-resistant C13 and CP70 cells, respectively. Similarly, serum deprivation induced significant docetaxel sensitivity in these cell lines. However, serum deprivation did not enhance docetaxel sensitivity in platinum-sensitive cells. Furthermore, serum deprivation did not have any effect on the sensitivities to cisplatin, vincristine, or doxorubicin in any of these cells. Increases in apoptotic cells of more than 700% were observed in C13 and CP70 cells when they were subjected to serum deprivation followed by exposure to paclitaxel. Serum deprivation decreased mitochondrial membrane potential ( $\Delta\Psi_m$ ) in C13 and CP70 cells. This finding indicates that serum deprivation induces depolarization specifically in platinum-resistant cells. These results indicate that serum deprivation confers hypersensitivity to taxane specifically in platinum-resistant cells by improving their impaired mitochondrial functions. This finding might be clinically beneficial for the development of new chemotherapeutic technology, particularly for patients with platinum-resistant ovarian cancer.

### 3. Cytokine gene expression signature in ovarian clear cell carcinoma

Cytokine expression in a tumor microenvironment can affect both host defense against the tumor and tumor cell survival. In this study, we sought to clarify whether the cytokine gene expression profile has clinical associations with ovarian cancer. We analyzed the expression of genes for 16 cytokines (interleukin [IL]-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p35, IL-12p40, IL-15, interferon  $\gamma$ , tumor necrosis factor  $\alpha$ , IL-6, HLA-DRA, HLA-DPA1, and colony-stimulating factor 1) in 50 ovarian carcinomas. Hierarchical clustering analysis of these tumors was performed with Cluster software, and differentially expressed genes were examined between clear cell carcinoma (CCC) and other subtypes. Following this examination we evaluated the biological significance of IL-6 knockdown in CCC. Unsupervised hierarchical clustering analysis of cytokine gene expression revealed 2 distinct clusters. The relationship between the 2 clusters and clinical variables showed statistically significant differences in CCC compared with other histologic types. The CCC showed a dominant Th-2 cytokine expression pattern driven largely by IL-6 expression. Inhibition of IL-6 in CCC cells suppressed signal transducer and activator of transcription 3 (Stat3) signaling and rendered cells sensitive to cytotoxic agents. The unique cytokine expression pattern found in CCC may be involved in the pathogenesis of this subtype. In particular, high IL-6 expression appears likely to be driven by the tumor cells, fueling an autocrine pathway involving IL-6 expression and Stat3 activation, and may affect survival when the tumor cells are exposed to cytotoxic chemotherapy. Modulation of IL-6 expression or its related signaling pathway is a promising treatment strategy for CCC.

### 4. Hypoxia promotes glycogen synthesis and accumulation in human ovarian CCC

Ovarian CCC has several significant characteristics based on molecular features that are distinct from those of ovarian high-grade serous carcinoma. Cellular glycogen accumulation is the most conspicuous feature of ovarian CCC, and in the present study its metabolic mechanism was investigated. The amount of glycogen in cells cultured under

hypoxic conditions increased significantly and approximately doubled after 48 hours ( $P < 0.01$ ) as compared with cells cultured under normoxic conditions. Periodic acid-Schiff staining also demonstrated intracellular glycogen storage. Western blot analysis revealed that hypoxia-inducible factor 1 $\alpha$ , which was overexpressed and stabilized under hypoxic conditions, led to an increase in the level of cellular glycogen synthase 1, muscle type (GYS1), and, conversely, to a decrease in inactive phosphorylated GYS1 at serine (Ser) 641. Additional increases were observed in both protein phosphatase 1, which dephosphorylates and thereby induces GYS1 enzyme activity, and glycogen synthase kinase 3 beta (GSK3 $\beta$ ) phosphorylated at Ser9, which does not act on phosphorylated GYS1 and subsequently induces its enzyme activity. In contrast, levels of phosphorylase, glycogen, muscle (PYGM)-b decreased. These results indicate that the glycogen accumulation in a hypoxic environment promotes glycogen synthesis but does not inhibit glycogen degradation or consumption. Under hypoxic conditions, HAC2 cells showed activation of the phosphatidylinositol 3-kinase (PI3K)/AKT pathway caused by a mutation in exon 20 of *PIK3CA*, encoding the catalytic subunit p110 $\alpha$  of PI3K. The resulting activation of AKT (phosphoSer473) also plays a role as a central enhancer in glycogen synthesis through suppression of GSK3 $\beta$  via phosphorylation at Ser9. Hypoxia decreased the cytotoxic activities of cisplatin and doxorubicin to various degrees. In summary, hypoxic conditions, together with the expression and stabilization of hypoxia-inducible factor 1, increased the intracellular glycogen contents and the resistance to anti-cancer drugs.

#### 5. Identification of a novel long noncoding RNA involved in the tumorigenicity of ovarian CCC

Ovarian CCC has a poor prognosis because of its chemoresistance. Therefore, identifying novel therapeutic targets is important. Recently, several long noncoding RNAs (lncRNAs), such as HOTAIR, have been reported to be aberrantly expressed in human cancers. However, lncRNAs involved in the tumorigenicity of ovarian CCC have not been reported. In this study, we attempted to identify novel lncRNAs that are required for the tumorigenicity of CCC. Apoptotic cell death was detected with the MEBCYTO Apoptosis Kit (MBL International, Woburn, MA, USA). Cell growth was measured with the methylthiotetrazol assay. For subcutaneous xenograft experiments, ovarian CCC JHOC5 cells infected with a lentivirus expressing a short hairpin RNA targeting lncRNA were injected stereotactically into 6-week-old nude mice. We found that knockdown of a novel lncRNA, termed ASBEL (antisense noncoding RNA in the BTG3 locus), resulted in a marked increase in the apoptotic death of JHOC5 cells. Moreover, knockdown of ASBEL significantly repressed the tumorigenicity of JHOC5 cells. We have identified a novel lncRNA, ASBEL, which is required for the tumorigenicity of CCC and, thus, is a promising target for the diagnosis and therapy of CCC.

#### 6. Development of second-generation photodynamic therapy for cervical cancer aiming at reducing photosensitivity and shortening length of stay

Uterine cervix conization has become a standard method of uterus-preserving therapy for early stage cervical cancer. However, because a 2006 article in *The Lancet* reported that conization increases the risks of premature birth, low birth weight, and cesarean delivery, the 2011 guidelines for the treatment of cervical cancer of the Japan Society of Gyneco-

logic Oncology states that informed consent concerning these risks is necessary before conization. On the other hand, photophrin photodynamic therapy (PDT) for cervical cancer shows a high complete response rate (97%) and low obstetrical risk, as mentioned above, but PDT is not recommended as the standard treatment for cervical cancer because it has the side effect of photosensitivity and requires a hospital stay as long as 3 weeks. Therefore, to develop second-generation PDT for cervical cancer with the aim of reducing photosensitivity and shortening hospital stays, we tested a semiconductor laser and its adaptation to the existing probe for uterine cervix irradiation in collaboration with Professor Kunio Awazu (Department of Engineering, Osaka University). First, we produced an FC adapter to connect a direct irradiation probe for lung cancer to an existing probe for uterine cervix irradiation. Then, we connected the direct irradiation probe for lung cancer to the main apparatus of the PD laser, and through the FC adapter connected an existing probe for uterine cervix irradiation to a probe for lung cancer. We performed laser irradiation under different experimental conditions. Next year, we plan to perform a phase I clinical trial of second-generation PDT using the photosensitizer talaporfin sodium (Laserphyrin, Meiji Seika Pharma Co., Ltd., Tokyo, Japan) with less photosensitivity.

### *Perinatology*

#### 1. Antiphospholipid antibodies and dysregulated clotting factors differentially affect the villous trophoblast in fetal growth restriction

Antiphospholipid antibodies (aPLs) and dysregulated clotting factors are associated with pregnancy pathologies, including preeclampsia and fetal growth restriction. Here we investigated the effect of aPLs and dysregulated clotting factors on the biology of the villous trophoblast. Placental samples were collected from healthy control subjects ( $n=8$ , 25–38 weeks) and patients (all with a small for gestational age infant) with aPLs/antiphospholipid syndrome (APS;  $n=10$ , 25–36 weeks) or dysregulated clotting factors ( $n=9$ , 27–35 weeks). Placentas were stained for Ki-67 and cytokeratin 7 to identify proliferating villous cytotrophoblast. Images were systematically and randomly selected, and Ki-67-positive cytotrophoblast and fibrin deposits were counted. There were no significant differences in gestational age at delivery, maternal age, or body-mass index. Fetal weight differed significantly between control subjects ( $2,291\pm 833$  g) and patients with APS ( $1,160\pm 413$  g) and between control subjects and patients with dysregulated clotting factors ( $1,182\pm 516$  g). In patients with APS, placental weight tended to be lower and the P/F ratio tended to be higher than in patients with dysregulated clotting factors. The percentage of Ki-67-positive cytotrophoblast in control subjects gradually decreased with gestational age, whereas this decrease was not apparent in patients with APS or dysregulated clotting factors. The relative number of Ki-67-positive cells was significantly lower in patients with APS ( $12.1\%\pm 7.1\%$ ) and dysregulated clotting factors ( $12.7\%\pm 5.8\%$ ) than in control subjects ( $22.6\%\pm 6.4\%$ ). The total number of cytotrophoblasts was lower in patients with APS ( $578\pm 209$ ) than in patients with dysregulated clotting factors ( $830\pm 258$ ) or in control subjects ( $780\pm 149$ ). There was no significant differences in total fibrin deposition or intravillous fibrin deposition. However, perivillous fibrin deposition was significantly greater in patients with dysregulated clotting factors

( $3.7\% \pm 1.5\%$ ) than in patients with APS ( $1.9\% \pm 1.1\%$ ). We suggest that aPLs downregulate cytotrophoblast proliferation throughout pregnancy, whereas in patients with dysregulated clotting factors, an increasing amount of fibrin deposition finally leads to a decrease in trophoblast proliferation but does not affect the total cell count.

## 2. Pathological changes in aborted tissue from patients who have had recurrent spontaneous abortions with aPLs induced by paternal lymphocyte immunization

Immunization with paternal lymphocytes for patients with recurrent spontaneous abortion (RSA) reportedly stimulates maternal immune response and transmits blocking antibodies and, therefore, contributes to the maintenance of pregnancy. However, this treatment may induce autoantibodies, including aPLs, that may be unfavorable for pregnancy. Seventy-one patients with RSA of unknown cause but without autoantibodies, including aPLs, underwent immunization with paternal lymphocytes at The Jikei University Hospital from April 2003 through December 2011. The presence of aPLs was assessed after treatment. Therapeutic outcomes were compared between patients in whom aPLs were induced and patients in whom they were not. We investigated pathological changes in aborted tissues from patients who miscarried after treatment. Of the 71 patients, 15 patients (21.1%) showed induced aPLs and 56 patients (78.9%) did not. Of the patients with induced aPLs, 14 had anticardiolipin immunoglobulin G antibodies. The pregnancy success rate was 63.6% (7 of 11 pregnancies) in patients with induced aPLs and 67.5% (27 of 40 pregnancies) in patients without induced aPLs. In patients with induced aPLs, anticoagulant therapy was performed for 7 patients, 6 of whom (85.7%) successfully maintained pregnancy; in contrast, of 4 patients who did not receive anticoagulant therapy, only 1 successfully maintained pregnancy (25%). Investigation of pathological changes in aborted tissues with a normal karyotype revealed characteristic changes in 4 of 7 cases in patients with induced aPLs, whereas no changes were observed in patients without induced aPLs (0 of 6 cases). Changes included abortive vessels; pink, amorphous deposits; and lymphocyte infiltration. After immunization with paternal lymphocytes the possible induction of aPLs should be assessed. Subsequent anticoagulant therapy may prevent abortion even under these adverse conditions.

## *Reproductive endocrinology*

We investigated potential clinical indicators of the outcome of in vitro fertilization (IVF) treatment in women 40 years or older undergoing infertility treatment. We retrospectively examined the results of IVF treatment in a total of 111 women 40 years or older. We found that in addition to patient age and the number of treatment cycles, cancellation of a treatment cycle could be a useful indicator of pregnancy outcome. Moreover, we investigated whether anti-Mullerian hormone would be a useful indicator for infertile women 40 years or older to consider ending infertility treatment. Our study indicated that anti-Mullerian hormone is not a definitive indicator to consider ending infertility treatment for infertile women 40 years or older.

In our next study, we investigated the relationship among outcomes of infertility treatment, ovarian reserve, and endometriosis in infertile women 40 years or older to establish an indicator for ending infertility treatment. Our results suggest that endometriosis decreases oocyte quality and is a useful predictor of treatment outcome in infertile women

40 years or older.

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## Department of Urology

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 Yasuyuki Suzuki, *Associate Professor*  
 Takashi Hatano, *Assistant Professor*  
 Akira Furuta, *Assistant Professor*

Shoichi Onodera, *Professor*  
 Isao Ikemoto, *Professor*  
 Koji Asano, *Associate Professor*  
 Nozomu Furuta, *Associate Professor*  
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 Takahiro Kimura, *Assistant Professor*

### General Summary

We performed research in the following areas: urologic oncology, urinary tract infection and sexual transmitted diseases, urodynamics and erectile dysfunction, the kidney and adrenal gland, endourology, and extracorporeal shockwave lithotripsy.

### Research Activities

#### *Urologic oncology*

1. Basic research: We performed several research projects to clarify the biology of urological malignancies and develop new therapeutic tools. Results were reported at the annual meetings of the Japanese Urological Association and the American Urological Association. The projects were as follows.

- 1) Proteomic analysis of new biomarkers for prostate cancer and urothelial cancer
- 2) Establishment and biological analysis of our new prostate cancer model, named JDCaP, derived from a Japanese patient
- 3) Research in neurourology and female urology

2. Clinical research: Several clinical studies are in progress in our department. Some works have already been reported at the annual meeting of the Japanese Urological Association.

- 1) Study of seeds and hormones for intermediate-risk prostate cancer, a phase III, multicenter, randomized, controlled trial
- 2) Clinical study of high dose rate brachytherapy with external beam radiation therapy for high-risk prostate cancer
- 3) Study of deep venous thrombosis after urological surgery
- 4) Study of the incidence of latent prostate cancer
- 5) Clinical study of preoperative image evaluation for patients undergoing radical prostatectomy
- 6) Clinical study of nomograms for predicting unilateral pathological T3 prostate cancer
- 7) Clinical study of 3-dimensional image construction of positive surgical margins in patients with prostate cancer
- 8) Clinical study of extended radical lymphadenectomy in patients with urological cancer

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- Sydes MR<sup>1</sup>, Egawa S, Sanders K<sup>1</sup>, Amos C<sup>1</sup>, Clarke N<sup>2</sup>, Kimura T, James ND<sup>3</sup> (<sup>1</sup>Med Res Council Clin Trials Unit, <sup>2</sup>Christie Salford Royal Hosp Found Trusts, <sup>3</sup>Univ Birmingham). Reflections on attempted Anglo-Japanese collaboration on STAMPEDE: a randomized controlled trial for men with prostate cancer. *Int J Urol*. 2011; **18**: 553-5.
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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 had been extracted, we were able to safely implant hydrophobic acrylic single-piece IOLs through a 1.8-mm incision. We are able to choose various premium IOLs, for example, multifocal IOLs, toric IOL, and yellow IOLs. We implant these new IOLs and evaluate subsequent visual function.

#### *Neuro-ophthalmology*

1. Cases of anti-aquaporin (AQP)-4 antibody-positive familial neuromyelitis optica (NMO) in mothers and daughters are described. The demographic, clinical, neuroimaging findings and the anti-AQP-4 antibody status were investigated in 4 patients from 2 Asian families with anti-AQP-4 antibody-positive NMO. NMO was diagnosed with the latest diagnostic criteria in both mothers and daughters. All patients were anti-AQP-4 antibody-positive. Disease onset occurred at different ages, even within the same family. These cases will enhance our understanding of the genetic contribution to NMO. Our findings suggest that familial history must be carefully examined in patients with NMO.
2. We presented the results of research on OPA1 and OPA3 gene mutations in 5 families with autosomal dominant optic atrophy, the estimation of Meyers' loop with diffusion

tensor imaging in a patient who had undergone temporal lobectomy, the imaging of the optic radiation with 3-T MRI in patients and healthy control subjects, and the clinical features and evaluation with 3-dimensional true fast imaging with steady-state precession and 3-dimensional time-of-flight MR angiography in patients with superior oblique myokymia.

- 1) Clinical features were reviewed in a textbook for residents of ophthalmology on trauma of the optic nerve, optic nerve sheath meningioma, sphenoid meningioma, and the visual sensory system in aging.
- 2) We lectured at a symposium on medical treatments for neuro-ophthalmic disorders.
- 3) We reported cases of atypical Leber's hereditary optic neuropathy associated with systemic vasculitis and lymphadenopathy, Leber's hereditary optic neuropathy preceded by bilateral idiopathic optic neuritis, unilateral papilledema with normal cerebrospinal fluid pressure, a limited form of NMO with a lesion of the trochlear nerve nucleus, and papilledema in a child. These reports described the etiologic relationship and were highly suggestive of pathogenesis.

#### *Ocular oncology and histopathology*

1. Orbital tumors metastatic from prostate carcinoma are common in Western countries but have a frequency of only 3.1% in Japan. Radiological findings and clinical course in a case of orbital metastasis from prostate carcinoma showing deterioration after improvement of hormonal therapy was reported. Malignant lymphomas are important in ocular oncology. However, natural killer (NK)/T-cell lymphomas constitute an extremely small fraction of ocular adnexal lymphomas. We reported 2 cases of NK/T-cell lymphoma in the ocular adnexa. Both cases had an aggressive clinical course, even with radiation and chemotherapy, and had a poor outcomes with multiple recurrences. The clinical course of NK/T-cell lymphomas in the ocular adnexa differs from that of mucosa-associated lymphoid tissue lymphomas. These cases provide insight about the clinical and pathological varieties of ocular adnexal lymphomas, and each subtype of malignant lymphoma requires prompt treatment.
2. The clinical and pathologic features of retinal hemangioma, tumors of the retinal pigment epithelium, retinal hamartoma, and tumors of the optic nerve were reviewed in a textbook for residents of ophthalmology.
3. We lectured at a symposium on the status and problem in surveys of ocular tumors and the significance of multicenter studies.

#### *Glaucoma*

1. The purpose of the treatment of glaucoma is to maintain visual function, and the lower intraocular pressure. 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 made possible detailed measurement of the 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).

2. Because eyedrops are needed for the long-term treatment of glaucoma, patient compliance is important. For drug therapy,  $\beta$ -adrenergic receptor antagonist eyedrops have been used. Twice-daily administration was necessary, but several kinds of eyedrops that can decrease intraocular pressure (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 ease of use in a patient with glaucoma. We found that the daily long-acting carteolol hydrochloride eyedrops improved compliance and were more convenient, and were equal to twice-daily eyedrops in lowering IOP.

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

### *Functional neuroimaging*

Patients with glaucoma were examined with voxel-based morphometry to confirm several structural changes in the visual pathway. The chiasm was evaluated with 3-dimensional T1-weighted images, and the structure of the optic radiation was evaluated with diffusion-tensor images. Both types of image were acquired with a clinical MR scanner. Fifteen patients with glaucoma and 15 age-matched healthy volunteers were

recruited. A significant signal decrease was observed with voxel-based morphometry in parts corresponding to the optic chiasm and the optic radiation in patients with glaucoma. Thus, these findings suggest that in glaucoma structural changes of the visual pathway occur in intracranial structures as well as in the eyeball.

#### *Developmental functional abnormality*

Binocular summation on the visual cortex was explored with fMRI in patients with post-operative 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. Review papers for visual psychology and neuro-ophthalmology

We wrote review papers that summarized contemporary topics about visual information processing in the primary visual cortex (V1), plasticity in the visual cortex, photophobia, visual experiences during dreaming, and cortical visual processes.

##### 2. Follow-up report for plasticity in adult human V1

To gather more information, we extended our measurements to subjects with retinitis pigmentosa. Our results were the same as in macular degeneration; there is no large-scale remapping in the adult human V1. Our results support vision-restoring therapies that rely on the stability of the human V1.

##### 3. Publishing 3 papers in international journals

Three of our research studies we reported previously here have been published: 1) Objective perimetry using fMRI (*Experimental Neurology*; impact factor=3.9); 2) Two temporal channels in human V1 identified using fMRI (*NeuroImage*; impact factor=5.7), and 3) Evaluation of subjective color sense after cataract surgery from the super early state (15 minutes after removing an eye patch) (*Journal of the Optical Society of America*; impact factor=1.9).

#### *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 of select cases that do not require the full capability of conventional vitrectomy.

To evaluate the clinical efficacy of the 7-mm IOL (Eternity®, Santen Pharmaceutical Co. Ltd., Osaka, Japan) for combined pars plana vitrectomy, phacoemulsification, and IOL implantation, we observed the visibility of the retina during vitrectomy and measured the depth of the anterior chamber preoperatively and postoperatively with the Pentacam®

scanner (Oculus Optikgeräte GmbH, Wetzlar, Germany).

We are planning to evaluate 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 is functional disorder retinal dystrophy, or 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 from 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, the stimulator can compare with visual field examination and is evaluated between subjective visual field examinations and objective ERGs. The color ERG records each response to separate long-, middle-, and short-wavelength cones. Recently, we have 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.

In the future, we will evaluate waveforms recorded from these ERG stimulators and analyze them with personal computer programs. Moreover, as we extract 1 waveform from 1 type of retinal cell, we will attempt to detect retinal disorders at a cellular level.

### *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 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.

The vulnerability of RGCs 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 reviewed findings of spectral domain OCT, fluorescein angiography, and indocyanine green angiography in patients with multifocal posterior pigment epitheliopathy treated with low-fluence photodynamic therapy (PDT). Early application of low-fluence PDT for multifocal posterior pigment epitheliopathy led to restoration of photoreceptor integrity with improvement of choroidal thickness and circulation.
2. We reported the findings of angiography and OCT evaluation of steroid-associated central serous chorioretinopathy in a patient with Vogt-Koyanagi-Harada disease. Accurate understanding of angiography findings is important for establishing diagnoses, although the noninvasive OCT provides helpful information.
3. We reported a case of refractory uveitis with bilateral optic disc swelling and retinal vasculitis that was suspected to be associated with idiopathic retinal vasculitis, aneurysms, and neuroretinitis.
4. We reported the outcomes of combined phacoemulsification and pars plana vitrectomy for restoring visual acuity in patients with cataract and posterior segment involvement due to ocular tuberculosis. Results indicate that combined phacoemulsification and pars plana vitrectomy can be used to remove cataracts and pathologic vitreous in the eyes of such patients. Although the exact role of vitrectomy remains to be determined, the combined surgery successfully restored useful vision in all cases.

### *Macular degeneration*

1. Single-session PDT combined with intravitreal bevacizumab and subtenon triamcinolone acetate for polypoidal choroidal vasculopathy  
We evaluated the efficacy of triple therapy consisting of single-session PDT, intravitreal bevacizumab, and subtenon triamcinolone acetate as the initial therapy for polypoidal choroidal vasculopathy. We found that this triple therapy improves vision and reduces central macular thickness in polypoidal choroidal vasculopathy.
2. We described findings in spectral-domain OCT for 4 patients with acute foveal photoreceptor damage. These 4 cases of acute foveal photoreceptor damage may represent a novel clinical entity. As more cases are recognized, the characteristic features of the disease spectrum and etiology may become clearer.

### *Biochemistry*

1. The peroxisome proliferator-activated receptor- $\alpha$  agonist fenofibrate has been shown to have anti-inflammatory activity and to suppress the development of experimental autoimmune encephalomyelitis. We investigated the effects of fenofibrate in experimental autoimmune uveoretinitis (EAU). The results suggest that fenofibrate modulates the development of EAU and suppress intraocular inflammation by decreasing the production of inflammatory cytokines.
2. Inhibition of extracellular signal regulated kinase (ERK) mitogen-activated protein kinase suppresses interleukin (IL)-17 production driven by IL-23- and IL-1 and attenuates autoimmune disease. We investigated the effects of the ERK inhibitors PD98059 and U0126 on EAU. The ERK inhibitors exhibited significant anti-inflammatory and immunosuppressive effects in EAU. Such ERK inhibitors are promising treatments for

autoimmune uveitis.

### *Color vision defects and genetic analysis of retinal diseases*

1. We investigated differences in color discrimination between the fellow eye and the affected eye successfully treated for unilateral age-related macular degeneration in a 69-year-old man with protanopia (a type of dichromacy in congenital color vision defects).
2. We performed clinical and molecular genetic analyses of various inherited retinal diseases, such as retinitis pigmentosa and macular and cone dystrophies. We identified causative mutations in these diseases. To clarify disease haplotypes, results of haplotype analysis of mutations were compared between family members and control subjects.
3. We investigated the involvement of various genetic factors in Japanese patients with age-related macular degeneration, a common cause of blindness in industrialized countries. More than 500,568 single-nucleotide polymorphisms of the whole genome were genotyped with Affymetrix Human Mapping Arrays and the TaqMan assay (Affymetrix Inc., Santa Clara, CA, USA). We are now analyzing candidate single-nucleotide polymorphisms involved in Japanese patients with age-related macular degeneration.

### *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 an imported donor cornea can be used according to the condition of the disease. We have performed Descemet's stripping automated endothelial keratoplasty for more than 30 patients and have obtained good postoperative results.

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## Department of Otorhinolaryngology

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 Nobuyoshi Otori, *Associate Professor*  
 Mamoru Yoshikawa, *Assistant Professor*  
 Yuichiro Yaguchi, *Assistant Professor*

Takakuni Kato, *Professor*  
 Hiromi Kojima, *Associate Professor*  
 Makoto Iida, *Assistant Professor*  
 Yoshinori Matsuwaki, *Assistant Professor*

### General Summary

Our basic and clinical studies have examined: the pathogenesis of cholesteatoma, surgery for adhesive otitis media, image-guided surgery with intraoperative computed tomography scan update, space motion sickness, nasal allergy, endoscopic endonasal sinus surgery (ESS), endoscopic endonasal skull-base surgery, sleep apnea syndrome, olfactory disorders, 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, operations for cholesteatoma 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 performing the genetic analysis of deaf patients in collaboration with Shinshu University.

We perform approximately 200 middle-ear operations 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 trans-mucosal gas exchange.

In the field of neuro-otology, we have introduced vestibular evoked myogenic potential (VEMP) testing to evaluate saccular function to facilitate diagnosis and treatment in patients with such conditions as vestibular neuritis, Meniere's disease, and dizziness of

unknown cause. 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 of having 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 ESS and from prospective studies of the post-operative course. We perform special care for skull-base conditions, such as pituitary tumors and cerebrospinal fluid leak, in close collaboration with the Department of Neurosurgery. We have reported case studies and investigated the postoperative course of diseases of the skull base. In an attempt to expand the indications for ESS from paranasal sinus tumors to skull-base surgery, including that for cerebrospinal 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 computed tomography scan update for an image-guided system to adapt 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 developed clinical studies and treatment methods for patients with a variety of olfactory disorders. We began the rehabilitation for patients with olfactory disorder for the first time in Japan. Since last year we have offered anatomy training using fresh-frozen cadavers in the Skills Laboratory, for training in both skull-base surgery and ESS. We must improve medical techniques and anatomical knowledge. In addition, we started creating new methods of Internet access using telemedicine and a distance-training system. To clarify the pathogenesis of chronic eosinophilic rhinosinusitis and allergic fungal rhinosinusitis, we investigated how environment fungi and bacteria induce activation and degranulation of human eosinophils.

#### *Research issues for 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 an 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 investigated the roles of surgery, such as uvulopalatopharyngoplasty. We will be able to present the adaptation of surgical treatment for sleep disorders. Long-distance sleep examinations have been performed since 2009 at the Ota Sleep Science Center.

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## Department of Anesthesiology

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 Masaki Kitahara, *Associate Professor*  
 Masamitsu Sanui, *Associate Professor*  
 Takahiro Matsumoto, *Assistant Professor*  
 Yoichi Kase, *Assistant Professor*

Sachiko Omi, *Professor*  
 Shuya Kiyama, *Associate Professor*  
 Yasushi Mio, *Associate Professor*  
 Sigehiko Uchino, *Associate Professor*  
 Yoshie Taniguchi, *Assistant Professor*  
 Kazuhiro Shoji, *Assistant Professor*  
 Hiroshi Sunaga, *Assistant Professor*

### General Summary

The 2011 academic year is the seventh year that the Department of Anesthesiology has been directed by Shoichi Uezono, M.D. 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 2011 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 2011.

### 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, neuro-anesthesia, obstetric anesthesia, intensive care medicine, and comprehensive pain management. Faculty recruitment is targeted at individuals with demonstrated academic and research activities as well as excellent clinical management and teaching skills. In 2011, the department was able to invite Takahiro Matsumoto, M.D., from Tsukuba University as an assistant professor. Doctor Matsumoto is a nationally recognized expert in teaching medicine, particularly with the aid of simulators. He is expected to promote the quality of our education program for residents and fellows. Doctor Hobo successfully finished a 2-year research fellowship at Wake Forest University in the United States and has rejoined our faculty. Our faculty and residents were both well represented at the Japanese Society of Anesthesiologists' annual meeting in Kobe and the American Society of Anesthesiologists' annual meeting in San Diego, California. In addition, members of the department continue to be invited as visiting professors or guest speakers at national and international meetings. Listed below are the ongoing research projects in which the principal investigators are faculty members of the Department of Anesthesiology.

Doctor Uezono's research focuses have been pulmonary vascular physiology and the

treatment of pulmonary hypertension associated with congenital heart disease. Doctor Hidano and Dr. Uezono retrospectively investigated maternal and neonatal outcomes in pregnant women undergoing cesarean section and found that pulmonary hypertension is a factor predicting maternal cardiac complications. 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 and has been extremely productive in the field of acute kidney injury.

Basic science investigations included studies of gene therapy for experimental pulmonary hypertension (Dr. Uezono), studies of the effects of sustained release of intrathecal morphine (Dr. Kondo), mechanisms of anesthetic postconditioning in myocardial mitochondria (Dr. Mio), gene polymorphism of vitamin D receptors in patients after cardiac surgery (Dr. Sanui), in-vivo nanoimaging techniques in cardiac disease (Dr. Terui), and prodrugs of opiates in the treatment of pain (Dr. Yasui).

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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### General Summary

Research topics of our department have focused on the following: 1) repetitive transcranial magnetic stimulation (rTMS) for stroke; 2) dysphagia after stroke; 3) development of the Kinder Infant Development Scale (KIDS); 4) diffusion tensor imaging in mild traumatic brain injury; and 5) effects of botulinum toxin injection for the upper limb after stroke.

### Research Activities

#### *rTMS for stroke*

1. A multicenter study of low-frequency rTMS combined with intensive occupational therapy for upper limb hemiparesis after stroke

The 15-day protocol of inpatient rTMS plus occupational therapy (OT) was confirmed as a safe, feasible, and clinically useful intervention for the affected upper limb after stroke in a large number of patients from different institutions. The response to treatment was not affected by age or time after stroke onset.

2. Outpatient application of rTMS and OT for upper-limb hemiparesis after stroke: A pilot study

Daily application of rTMS and intensive OT at an outpatient clinic is a novel treatment for patients with mild upper-limb hemiparesis after stroke.

3. Application of combined 6-Hz primed low-frequency rTMS and intensive OT for upper-limb hemiparesis after stroke

The 15-day protocol of 6-Hz-primed low-frequency rTMS combined with intensive OT seems to be a safe and useful treatment for upper-limb hemiparesis after stroke.

4. Therapeutic application of 6-Hz-primed low-frequency rTMS combined with intensive speech therapy for poststroke aphasia

The protocol of 6-Hz-primed low-frequency rTMS and intensive speech therapy for poststroke aphasia was safe and feasible, suggesting its usefulness in the treatment of this condition.

5. Combination treatment of low-frequency rTMS and OT with levodopa administration: An intensive neurorehabilitative approach for upper-limb hemiparesis after stroke

The combination treatment of low-frequency rTMS, intensive OT, and oral administration of levodopa could provide a safe and feasible intervention for upper-limb hemiparesis after stroke.

6. Antispastic effect of low-frequency rTMS and OT for patients with upper-limb hemi-

paresis after stroke

The 15-day inpatient protocol of low-frequency rTMS and intensive OT is potentially suitable for reducing spasticity and for improving the motor function of the affected upper limb after stroke.

7. Baseline severity of upper-limb hemiparesis affects the outcome of low-frequency rTMS and intensive OT in patients after stroke

The 15-day protocol of low-frequency rTMS and intensive OT is a promising treatment for improving the motor function of the affected upper limb. The extent of motor improvement seemed to be affected by the severity of upper limb hemiparesis at study entry.

8. A comparison of the effects of high- and low-frequency rTMS on upper-limb hemiparesis in the early phase of stroke

High-frequency rTMS applied to the lesional hemisphere in the early phase of stroke was more beneficial for motor improvement of the affected upper limb than was low-frequency rTMS.

#### *Dysphagia after stroke*

1. Swallowing analysis for semisolid food texture in patients with dysphagia after stroke  
The textures of different semisolid foods were identified. Adhesiveness and gumminess seemed to be related to residue deposition and aspiration.

2. Which cortical area is related to the development of dysphagia after stroke? A single photon emission computed tomography study using novel analytic methods

With single photon emission computed tomography and a novel method of analysis, regional cerebral blood flow in Brodmann areas 4 and 24 were found to be significantly lower in patients with dysphagia. In area 4, 80% sensitivity and 60% specificity for discriminating dysphagia were achieved with an optimal cutoff value.

#### *Development of the family-rated KIDS*

1. Evaluation of the family-rated KIDS for disabled children

We tested the validity and reliability of the KIDS rated by families for assessing disabled children. The results showed good validity and almost perfect reliability.

#### *Diffusion tensor imaging in mild traumatic brain injury*

1. Voxel- and atlas-based analyses of diffusion tensor imaging may reveal focal axonal injuries in mild traumatic brain injury: Comparison with diffuse axonal injury

Voxel- and atlas-based analyses of diffusion tensor imaging suggest that patients with mild traumatic brain injury have focal axonal injury and that the pathophysiology is significantly different from that of diffuse axonal injury. These findings will aid the diagnosis of patients with mild traumatic brain injury.

#### *Effects of botulinum toxin injection for the upper limb after stroke*

1. Injection of botulinum toxin type A followed by home-based functional training for upper limb hemiparesis after stroke

Our proposed protocol of injection of botulinum toxin type A followed by home-based

functional training might improve the active motor function of the affected upper limb after stroke.

## Publications

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**Hashimoto K, Mastui N, Yukawa H, Miyamura K.** Evaluation of the family-rated Kinder Infant Development Scale (KIDS) for disabled children. *Jikeikai Med J*. 2012; **59**: 5-10.

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## Department of Emergency Medicine

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 Kenji Okuno, *Assistant Professor*

Tsutomu Koyama, *Professor*  
 Satoshi Takeda, *Assistant Professor*  
 Taro Nameki, *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 heart attack 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

### 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 traumatic intracranial hypotension C.S.F (cerebo-spinal fluid)
8. Management of Japan Advanced Trauma Evaluation and Care Course

### Publications

Shigemori M<sup>1</sup>, Abe T<sup>1</sup>, Aruga T<sup>1</sup>, Ogawa T<sup>1</sup>, Okudera H<sup>1</sup>, Ono J<sup>1</sup>, Onuma T<sup>1</sup>, Katayama Y<sup>1</sup>, Kawai N<sup>1</sup>, Kawamata T<sup>1</sup>, Kohmura E<sup>1</sup>, Sakaki T<sup>1</sup>, Sakamoto T<sup>1</sup>, Sasaki T<sup>1</sup>, Sato A<sup>1</sup>, Shiogai T<sup>1</sup>, Shima K<sup>1</sup>, Sugiura K<sup>1</sup>, Takasato Y<sup>1</sup>, Tokutomi T<sup>1</sup>, Tomita H<sup>1</sup>, Toyoda I<sup>1</sup>, Nagao S<sup>1</sup>, Nakamura H<sup>1</sup>, Park Y<sup>1</sup>, Matsumae M<sup>1</sup>, Miki T<sup>1</sup>, Miyake Y<sup>1</sup>, Murai H<sup>1</sup>, Murakami S<sup>1</sup>, Yamaura A<sup>1</sup>, Yamaki T<sup>1</sup>, Yamada K<sup>1</sup>, Yoshimine T<sup>1</sup> (Jpn Neurosurg Soc). Guidelines for the management of severe head injury, 2nd edition, guidelines from the guidelines committee on the management of severe head injury, the Japan society of neurotraumatology. *Neurol Med Chir (Tokyo)*.

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**Ogawa T.** Vertigo and associated symptoms

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## Department of Endoscopy

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### General Summary

The main theme of our research is clinical studies using endoscopy in the diagnosis and treatment of gastrointestinal, hepatobiliary, and pancreatic disease. In addition, we perform basic research for developing novel instrumentation, image processing and analysis methods, as well as optical apparatuses, such as autofluorescence imaging (AFI), 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 of 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 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 is particularly important when screening patients from the non-referral hospital population, because it will reduce discomfort during endoscopic examination.

##### 1) Magnifying endoscopic observation using an NBI system

This new diagnostic system consists of a magnifying ( $\times 90$ ) endoscope and an NBI light source, which provides detailed morphological information about the capillaries on the mucosal surface. We studied the clinical utility of NBI magnifying endoscopy for superficial neoplasms of the pharynx, esophagus, stomach, and duodenum. One of the studies 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. Moreover, 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 precancer-

ous changes in the specialized columnar epithelium of Barrett's esophagus. Results of these studies have been reported at several conferences and published. Most recently, a magnifying endoscope and an NBI light source have been developed and become available for clinical use. We performed a study comparing NBI magnifying endoscopy and conventional high-definition magnifying endoscopy for detecting superficial carcinoma of the pharynx and esophagus.

## 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 premalignancies 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.

## 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 lesions 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  
Current indications for endoscopic mucosal resection (EMR) are limited by lesion size, depth, and histological type. Our recent efforts have focused on expanding the indications for endoscopic submucosal dissection (ESD) in the treatment of early gastric cancer, on the basis of histopathological findings. We are also evaluating the potential new use of EMR for gastric cancers, including small, poorly differentiated adenocarcinomas lacking ulceration, well-differentiated adenocarcinomas 30 mm or smaller or confined to the mucosa, and carcinomas lacking submucosal microinvasion. Current indications for EMR include esophageal cancer, epithelial cancer (m1), and cancer partially invading the lamina propria mucosae (m2) with a negligible risk of lymph-node metastasis. New indications for EMR now being evaluated include mucosal cancer invading the lamina muscularis mucosae (m3) and lesions with slight submucosal invasion within the inner third of the submucosal layer (sm1). At present, en bloc resection with ESD is considered necessary to further develop the use of endoscopic treatment. A new series of endoscopic knives and long-lasting submucosal fluid have successfully reduced 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. A study to evaluate the risks of sepsis and endotoxemia after ESD, using blood culture, is currently under way.

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. Studies using an M-scope with magnifying capability are now under way to develop more accurate and safer procedures. Furthermore, clinical studies using a newly developed therapeutic endoscope (R-scope), which 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 transluminal endoscopic surgery (NOTES), 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 also have 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 on 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 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 identify. Establishing methods to evaluate hypersensitivity and dysmotility of the gastrointestinal tract are important for understanding disease pathophysiology and 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 are 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 gastrosplenic shunts, which can complicate the treatment of esophagogastric varices, and can delineate shunts in detail. Therefore, this diagnostic system could 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 Western countries and 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 6 other hospitals 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 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 in the small intestine which have been unreachable with traditional push-type enteroscopy. Recently, particularly in the 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, which was published in scientific journals, 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 examining biological markers of

malignancy in sessile serrated lesions by means of immunohistochemical staining to evaluate whether such these lesions have malignant potential.

### *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. Additionally we introduced second-generation contrast media for ultrasonic imaging in the EUS diagnosis of pancreaticobiliary diseases.

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 with 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 digestive tract and bile duct tumors, we have performed endoscopic ballooning/bougienage and subsequent metallic stenting, with 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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Seiji Hori, *Professor*  
Hiroshi Takeda, *Assistant Professor*

Masaki Yoshida, *Assistant Professor*  
Yasushi Nakazawa, *Assistant Professor*

### General Summary

Our clinical research has clarified the characteristics of infectious diseases to facilitate accurate diagnosis and early treatment. In addition, we have demonstrated that active intervention by the Infection Control Team (ICT) can reduce the transmission of methicillin-resistant *Staphylococcus aureus* (MRSA). On the other hand, our studies of biofilm formation will clarify the mechanism of refractory bacterial infection. Thus, our investigations will lead to more effective therapies for infectious diseases and help prevention of the spread of infections.

### Research Activities

#### *Clinical studies of extended-spectrum $\beta$ -lactamase-producing Enterobacteriaceae isolated from urine*

We studied the clinical features of 78 patients with extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae isolated from urine. The most frequently isolated organism was *Escherichia coli*. Of the patients, 32 were outpatients, and among the patients who were hospitalized, 12 had community-acquired infections and 34 had hospital-acquired infections. Of the patients with community-acquired infection, 11 had been admitted from nursing homes or other hospitals. Indwelling urinary catheters had been present in 24 patients with hospital-acquired infection. Initial treatments failed in 57.1% of patients with febrile urinary tract infection and in 41.4% of patients with afebrile urinary tract infection. The resistance rate of *E. coli* to levofloxacin was 79.5%, but that to fosfomycin was only 11.0%.

#### *Evaluation of the role of the ICT in MRSA transmission*

The ICT was established 2008 and started a standard precaution campaign and repeated role-playing education in 2009. The ICT made efforts in the education of standard precaution practices in The Jikei University Hospital and examined the use of alcohol hand solution gloves, and gowns and the incidence of MRSA (newly positive cases 48 hours after admission/total patient days). We investigated the effects of our educational approach on controlling infections with MRSA. Since the private rooms are not enough in our hospital, all the patient who acquired MRSA were not isolated in private room other than the patient who is low activity of daily living. From April 1, 2007, to December 31, 2010, the yearly usage rates of alcohol hand solution and gowns in hospital increased from 2.99 to 5.03 and from 0.61 to 1.39, respectively. In contrast, the hospital-wide incidence of MRSA decreased from 0.65 to 0.47. These results suggest that the improvement in standard precaution compliance reduces hospital-wide MRSA transmis-

sion, although the incidence of MRSA is higher in Japan than in European countries.

*Risk factors for mortality in patients with bacteremia due to Pseudomonas aeruginosa*

We performed retrospective analyses to determine risk factors for mortality among patients with bacteremia caused by *P. aeruginosa*. A total of 134 patients with *P. aeruginosa* bacteremia were identified from April 2003 through March 2010. The 30-day mortality rate among all patients with *P. aeruginosa* bacteremia was 20.9%. This study revealed that factors indicating a poor prognosis were thrombocytopenia and polymicrobial *P. aeruginosa* bacteremia. On the other hand, mortality was not affected by inappropriate initial empirical antimicrobial treatment.

*Vertebral osteomyelitis due to biofilm producing Staphylococcus epidermidis*

We reported a case of spondylitis due to *S. epidermidis*. Biofilm forming assay revealed this *S. epidermidis* produced biofilms on polystyrene surfaces in broth medium. We found that other biofilms of *S. epidermidis* were susceptible to a polysaccharide-degradative enzyme. These findings suggest that polysaccharide is a major matrix adhesin in *S. epidermidis* biofilms.

*Clinical characteristics of the patients with primary human immunodeficiency virus infection*

We reviewed cases of primary human immunodeficiency virus (HIV) infection in our hospital and analyzed their clinical characteristics. Ten patients were included in this study. Frequent symptoms were fever, pharyngitis, lymphadenopathy, and skin eruption. Common laboratory abnormalities were neutropenia, thrombocytopenia, and elevated serum levels of aminotransferases. Acquired immunodeficiency syndrome developed in only 2 patients, who showed esophageal candidiasis and pneumocystis pneumonia. Patients with primary HIV infection, who are at high risk of transmission, greatly affect public health. We must recognize that patients with infectious mononucleosis-like symptoms must be examined serologically for HIV and that early diagnosis of HIV infection is important in high-risk groups.

*Evaluation of the transmission route of Entamoeba histolytica in patients with amoebic dysentery in our hospital*

We reviewed 19 patients with amoebic dysentery in The Jikei University Hospital from January 1, 2006, to December 31, 2010. Eighteen of the 19 patients were men, and the mean age was 45.8 years. Thirteen patients had amoebic colitis, 2 patients had amoebic liver abscesses, and 1 patient had amoebic liver and brain abscesses. Routes of transmission were sexual contact in 7 patients and travel abroad in 4 patients. For the infection control of sexually transmitted diseases, safer sex with condoms is important. Moreover, a greater emphasis should be placed on sexually transmitted diseases, such as amoebic dysentery, that are acquired via the fecal-oral route.

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- Hori S.** The appropriate antibiotics treatment for elderly persons, pregnant women and the patients with liver or renal damage (in Japanese). *Shindan to Chiryō*. 2012; **100**: 367-73.
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## Department of Dentistry

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Masashi Sugisaki, *Professor*  
Katsuhiko Hayashi, *Associate Professor*

Akihiro Ikai, *Associate Professor*  
Shigeru Suzuki, *Assistant Professor*

### General Summary

#### 1. Morphological and histological studies of the temporomandibular joint

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

#### 2. Clinical studies of temporomandibular disorders

We continued our studies to investigate, by means of a screening questionnaire, the relationship between temporomandibular disorders (TMDs) and sex, age, and job content.

### Research Activities

#### *Morphological and histological studies of TMJ*

##### 1. Anatomical notes for TMJ arthroscopy

The TMJ arthroscopy technique was developed by Ohnishi in 1970. Thereafter, many clinical and basic reports regarding the safety of TMJ arthroscopy have been published. The possible complications of the surgery, however, should not be forgotten. We reported on the anatomical characteristics of the TMJ for the express purpose of arthroscopy. Because many important organs are present around the TMJ, better knowledge of anatomical characteristics will help avoid severe complications, such as injury to the cranium, tympanic membrane perforation, and laceration of the external auditory canal. Therefore, the dissection of fresh cadavers is important. We reported results of fresh-cadaver dissection around the TMJ and described a safe insertion technique for arthroscopy.

#### *Clinical studies of TMDs*

##### 1. A pilot questionnaire study of the relationship between descriptions of job contents by sex and screening for TMDs in dental patients at general dental offices in Tokyo

We previously reported the results of a screening questionnaire regarding the prevalence and contributory factors of TMDs for persons working in Tokyo. Multivariate logistic regression analysis revealed that significant contributory factors for TMD in men were a feeling of fatigue (odds ratio [OR]=1.55) and in women were feelings of depression (OR=1.37) and fatigue (OR=1.30). The purpose of this pilot study was to investigate sex-related concerns between job contents and TMD. With the cooperation of the Tokyo Dental Association, we performed a questionnaire survey for applicants of dental check-ups at 13 general dental offices in Tokyo. Responses were obtained from 253 subjects and were used as secondary data for analysis. Because we excluded subjects who replied that their commuting time was 0, a total of 180 subjects were included. A questionnaire included 4 TMD screening questionnaire items, sex, age, and 9 job-content

questions, and the answers were subjected to multivariate logistic regression analysis. We found that mean age did not show sex differences. Regarding differences in job contents by sex, men had significantly longer driving time and meeting time, and women had a significantly longer time before going to bed (correction value:  $p=0.05/9=0.0062$ ). The personal computer (PC) operation time did not differ between sexes. Job contents differed between subjects who had TMD and those who did not. Multivariate logistic regression analysis showed that PC operation time was significant ( $p=0.031$ ;  $OR=1.85$ ) only for women. These findings suggest that sex and age affect the relationship between TMD and job contents. Although no direct relation was confirmed, using a PC at work was identified as contributory factor for TMD in women.

## 2. An evaluation of symptoms at initial visit in patients with TMDs: Comparison between patients younger than 45 years and patients 45 years or older

The frequency of osteoarthritis and sensibility for pain in geriatric patients with TMD are said to differ from those in young or middle-aged patients. To clarify the pathology of TMD between geriatric patients and young or middle-aged patients, we examined the frequency of TMD symptoms in geriatric patients at the first examination and compared the findings to those in young or middle-aged patients. At the first examination, the frequency of TMJ pain when the chin was pressed and tenderness of the masticatory muscles differed between the patient groups. Logistic regression analysis showed that the TMJ pain when the chin was pressed was less ( $OR=0.574$ ) and that the tenderness of the masticatory muscles was greater ( $OR=1.832$ ) in geriatric patients. The results show that the sensibility for pain of the TMD at the first examination differs between geriatric patients and young or middle-aged patients.

## 3. Pathology and treatment of TMDs in geriatric patients

To clarify the pathology of TMDs in geriatric patients, we examined the frequency of tenderness of the TMJ and of the masticatory muscles and the relationship between missing molars and condylar head abnormalities at the first examination, assessed the efficacy of treatment, and compared the findings to those in young or middle-aged patients. The results were as follows. At the first examination, the frequency of TMJ tenderness was high both in young or middle-aged patients and in geriatric patients. The frequency of TMJ pain when the chin was pressed and of tenderness of the masticatory muscles differed between the patient groups. Logistic regression analysis showed that the TMJ pain when the chin was pressed was less and that the tenderness of the masticatory muscles was greater in geriatric patients. The frequency of condylar head abnormalities was greater in geriatric patients than in young or middle-aged patients. The high frequency of condylar head abnormalities in patients with missing molars suggests that occlusion is a factor in osteoarthritis. Assessment of the efficacy of treatment for the same types of TMJ disorder showed that conservative treatment, mainly guidance in regard to diet and daily life, was equally effective for geriatric patients and for young or middle-aged patients.

## Publications

**Sugisaki M, Takano N<sup>1</sup>, Hayashi K, Saito T, Kuruma E, Kino K<sup>2</sup>, Nishiyama A<sup>2</sup>** (<sup>1</sup>*Tokyo Dent Assoc*, <sup>2</sup>*Tokyo Med Dent Univ*). A pilot study on the relationship between descriptions of job contents by gender and screening for temporomandibular disorders in dental patients at general clinical offices in metropolitan Tokyo using a questionnaire (in Japanese). *Nihon Gakukansetsu Gakkai Zasshi*. 2011; **23**: 143-8.

**Tsukagoshi K<sup>1</sup>, Nishiyama A<sup>1</sup>, Kino K<sup>1</sup>, Sugisaki M, Haketa T<sup>1,2</sup>** (<sup>1</sup>*Tokyo Med Dent Univ*,

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## Reviews and Books

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## Department of Transfusion Medicine

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Tetsunori Tasaki, *Professor*

Yoko Kato, *Assistant Professor*

### General Summary

1. To implement appropriate and immediate action after transfusion-related adverse events, a preliminary online hemovigilance system was developed in 2007. As of December 31, 2011, a total of 56 hospitals were participating in the system.
2. In Japan, red-cell concentrates manufactured in blood centers using the additive solution mannitol-adenine-phosphate are required to be used within 3 weeks, although the shelf life had been 6 weeks until 1995. The main reason for the change was concern over contaminating bacteria, such as *Yersinia enterocolitica*, which could grow even at low temperatures (4°C). On the other hand, the demand for blood usage has gradually increased with an ageing society and a low birthrate. These changes have also decreased the number of blood donors. The situation has put further pressure on efforts to maintain an adequate blood supply. Therefore, extension of the storage period is our focus, i.e., the shelf life of packed red cells should be increased to 6 weeks as before. To resolve the issue, the risk of bacterial contamination of stored blood derived from donors with symptoms of infectious disease was estimated accurately, taking cost-effectiveness into account.
3. Recently, several departments of transfusion medicine have engaged in so-called cell therapy in addition to routine transfusion testing or management of blood products. In our hospital, we have collected mononuclear cells with cell separators from patients with Buerger's disease, or arteriosclerosis obliterans, for use in their treatment. The close cooperation of specialists between transfusion medicine and clinical departments is essential for the development of cell therapy, including this vascular regeneration therapy.
4. In 1975, the World Health Assembly passed a resolution recommending the following: (1) whole-blood donation and supplementary plasmapheresis should be voluntary and unpaid, and (2) nations should try to become self-sufficient in blood and blood products. In 1983, however, one-third of plasma prepared in the world was used in Japan. Around that time, transmission of human immunodeficiency virus through unheated blood products became a subject of public concern. In the revised Act on Securing a Stable Supply of Safe Blood Products in 2002, the importance of self-supplying blood products by voluntary unpaid blood donors was clearly prescribed. Every hospital was required to make efforts to achieve this aim.
5. Although the quality and safety of allogeneic blood is extremely high, preoperative autologous blood donation is the norm in Japan for patients scheduled to undergo surgery requiring blood transfusion and for patients who prefer to use their own blood. The guidelines for autotransfusion are well established, but one unresolved issue is the suitability of donating autologous blood immediately after X-ray examination using a contrast medium.
6. Education in the best practices of transfusion medicine should be done for all medical

staff in the hospital, including medical students. Insufficient teaching staff is a problem in our hospital and is a common issue in departments of transfusion medicine in university hospitals in Japan.

### Research Activities

1. According to data collected in 2011 from the online reporting system, the overall incidence of adverse events per transfusion bag was 1.02% (5,068 of 494,914). The incidence of adverse events was significantly higher for platelet concentrates (2.46%) than for red blood cells (0.53%) or fresh-frozen plasma (0.66%).
2. Because bacterial contamination in blood components is a serious problem, its incidence is being carefully investigated for various types of blood products to justify the extension of the storage period from 3 weeks to 6 weeks. This work is being supported by Grants-in-Aid for Scientific Research. In 2011, an accurate frequency of incidents was not obtained because the blood samples tested were not appropriately collected or stored. Improved evaluation is planned next year for blood specimens obtained from patients with mild fever or diarrhea or both.
3. From June 2006 through May 2010, 6 patients with Buerger's disease, or arteriosclerosis obliterans, were treated with their own peripheral hematopoietic stem cells. Four of them showed improvements in skin color, pain, and other signs and symptoms. This result was reported at the 59<sup>th</sup> annual meeting of the Japan Society of Transfusion Medicine and Cell Therapy.
4. Fever or urticaria is frequently encountered during the transfusion of blood products. Especially in patients who previously experienced severe adverse events, antihistamines or corticosteroids or both are administered before transfusion to prevent such reactions. In our retrospective study, the pretransfusion treatment was effective in patients receiving plasma-rich products, such as fresh-frozen plasma or platelet concentrates. However, no benefit was demonstrated when red blood cells were transfused. More thorough investigations should be done to determine the appropriate use of such drugs to prevent transfusion-related adverse events. Our findings were reported at the 59<sup>th</sup> annual meeting of the Japan Society of Transfusion Medicine and Cell Therapy.
5. We evaluated the quality of gadolinium-contaminated blood in terms of the degree of hemolysis, production of microaggregates, and red blood cell shape. Our findings suggest that the presence of such a contrast medium in blood is unlikely to induce deleterious effects on blood components.

### Reviews and Books

**Kato Y, Kamitamari A<sup>1</sup>, Tajima A, Tamaki H, Itoh F, Hoshi Y, Kaneko T<sup>2</sup>, Ida H** (<sup>1</sup>*Sasebo City Hosp,* <sup>2</sup>*Tokyo Metropolitan Child Med Ctr*). Challenges in the management of an infant with

severe hemophilia A and intracranial hemorrhage. *Nihon Syoni Ketsueki Gakkai Zasshi.* 2011; **25**: 130-4.

## Institute of DNA Medicine

### Department of Gene Therapy

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Toya Ohashi, *Professor and Director*

Hiroshi Kobayashi, *Associate Professor*

#### General Summary

##### *Study of antibody formation during enzyme replacement therapy for Fabry disease*

Two enzyme preparations are available for enzyme replacement therapy for Fabry disease. One is agalsidase alfa ( $\alpha$ ), and the other is agalsidase beta ( $\beta$ ). The antibody against  $\beta$  cross-reacted with  $\alpha$  to the same extent as to  $\beta$ . The anti- $\beta$  antibody neutralizes the enzyme activity of both  $\alpha$  and  $\beta$  equally and inhibited the cellular uptake of  $\beta$ . The antibody titer assayed with enzyme-linked immunosorbent assay was positively correlated with the neutralizing activity of  $\beta$  and the inhibition of cellular uptake by  $\beta$ .

##### *Neonatal gene therapy for Krabbe disease*

We have studied the therapeutic effects of gene therapy in the neonatal mouse model of Krabbe disease, a progressive demyelinating disease. We injected a recombinant lentiviral vector including an enzyme (galactocerebrosidase) expressing the gene for the mouse neonatal facial vein and detected the significant effects of reduced substrate accumulation, improved pathological findings, and increased life span. We are preparing studies of more efficient transduction, *ex-vivo* gene transfer, and a homologous recombination system using the zinc finger method.

##### *Pathophysiological analysis of Pompe disease*

We analyzed the signaling pathway of endoplasmic reticulum stress-independent autophagy in fibroblasts derived from patients with Pompe disease. We found decreased levels of phosphorylated Akt and phosphorylated p70 S6 kinase in the fibroblasts. This result suggests that the down-regulated Akt/mammalian target of rapamycin pathway activates autophagy in fibroblasts from patients with Pompe disease.

##### *Antitumor effect and application to gene therapy of nafamostat mesilate for fatal digestive cancer*

Recent studies have demonstrated that nuclear factor (NF)- $\kappa$ B plays an important role in the regulation of cell apoptosis, inflammation, and oncogenesis. Inhibition of NF- $\kappa$ B is a potential new strategy for the treatment of cancers. We have previously reported that nafamostat mesilate, a serine-protease inhibitor, inhibits NF- $\kappa$ B activation and induces the apoptosis of pancreatic cancer. Moreover, we have shown that the addition of nafamostat mesilate promotes apoptosis induced by gemcitabine or paclitaxel owing to the inhibition of the NF- $\kappa$ B activation of pancreatic cancer. The clinical usefulness of the combination of gemcitabine and nafamostat mesilate for patients with unresectable pancreatic cancer was examined in a phase II study. Recently, we investigated the antitumor effects of nafamostat mesilate against other digestive cancers. Intraperitoneal combina-

tion therapy with paclitaxel and nafamostat mesilate enhanced the antitumor effect of paclitaxel in a mouse model of gastric cancer with peritoneal dissemination.

Gene therapy using an adenoviral vector expressing tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is a new therapeutic approach for chemoresistant malignancies. However, the efficacy of TNF- $\alpha$  is limited because of the activation of NF- $\kappa$ B. We hypothesized that the addition of nafamostat mesilate would enhance the antitumor effect of TNF- $\alpha$  gene delivery, and we have demonstrated the efficacy of the combination therapy against pancreatic cancer. Recently, we have investigated the efficacy of the combination therapy against hepatocellular carcinoma.

#### *Islet biology and molecular medicine in diabetes*

To develop a method for in-vitro observation of isolated islets of the pancreas, we performed animal experiments this year with a completed intercellular matrix. The results were submitted to a journal for publication.

As a clinical research, we performed study on pathophysiology of hypoglycemia by analyzing the timing of spontaneous hypoglycemia and the glucagon response with the continuous glucose monitoring in a patient with frequent hypoglycemia with unknown cause. From the hormone response, we hypothesized the patient should have impairment in the process of gluconeogenesis, and have started genetic analysis of candidate genes for the gluconeogenic enzymes including phosphoenolpyruvate carboxykinase, pyruvate kinase, and fructose-1,6-bisphosphatase.

#### *High-risk ovarian cancer based on 126-gene expression signature is uniquely characterized by downregulation of the antigen-presentation pathway*

High-grade serous ovarian cancers are heterogeneous both in terms of clinical outcomes and at the molecular level. Our aim was to establish a novel risk-classification system based on gene expression signatures for predicting overall survival which we hope will lead to novel therapeutic strategies for high-risk patients. In this large-scale cross-platform study of 6 microarray data sets from 1,054 patients with ovarian cancer, we developed a gene expression signature for predicting overall survival by applying elastic net and 10-fold cross-validation to Japanese data set A (n=260) and evaluated signatures in 5 other data sets. Subsequently, we investigated differences in the biological characteristics between patients with high- and low-risk ovarian cancers. An elastic net analysis of Japanese data set A identified a 126-gene expression signature for predicting overall survival in patients with ovarian cancer (multivariate analysis,  $P=4 \times 10^{-20}$ ). We validated the predictive ability of the signature through multivariate analysis with 5 other data sets (Tothill's data set,  $P=1 \times 10^{-5}$ ; Bonome's data set,  $P=0.0033$ ; Dressman's data set,  $P=0.0016$ ; TCGA data set,  $P=0.0027$ ; and Japanese data set B,  $P=0.021$ ). Through gene ontology and pathway analyses, we identified a significant reduction in expression of immune-response-related genes, especially on the antigen-presentation pathway, in patients with high-risk ovarian cancer. This risk classification based on the 126-gene expression signature is an accurate predictor of clinical outcome in patients with advanced-stage high-grade serous ovarian cancer and might lead to new therapeutic strategies for patients with high-grade serous ovarian cancer.

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## Institute of DNA Medicine

### Department of Oncology

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Mikio Zeniya, *Professor*  
Shigeo Koido, *Associate Professor*

Sadamu Homma, *Associate Professor*  
Yasuharu Akasaki, *Assistant Professor*

#### General Summary

Establishing effective treatments for malignant tumors on the basis of antitumor immune responses is a goal of this department. For this purpose, several experimental and clinical studies of cancer immunotherapy and cancer vaccines have been performed. Promising results were obtained from clinical studies of cancer vaccines targeting advanced pancreatic cancer and glioblastoma. A unique artificial protein generated with motif-programming technology could become a next-generation cancer vaccine for inducing potent antitumor immunity. Novel cancer-specific antigens that might be ideal targets for antitumor immune responses were found with mass spectrometric analysis. Induced pluripotent stem (iPS) cells have been used to generate new cancer vaccines. For cancer vaccine therapy, antigenic peptides of known tumor antigens can be detected with mass spectrometric analysis of formalin-fixed tumor tissue.

#### Research Activities

##### *Phase I and II clinical studies of Wilm's tumor protein 1-targeting immunotherapy against advanced pancreatic cancer*

Because pancreatic cancer is a devastating disease with an extremely poor prognosis, establishing effective treatments for pancreatic cancer is an urgent issue. We have started a phase I clinical study of combined therapy with gemcitabine and dendritic cells pulsed with Wilm's tumor protein (WT) 1 class I and II peptides in collaboration with the Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kashiwa Hospital. To our knowledge, this study is the first of vaccination with dendritic cells pulsed with both WT1 class I and class II peptides for treating pancreatic cancer. However, a phase I clinical study of combination therapy with gemcitabine and a WT1 peptide vaccine has been completed, and its results are now being analyzed. Adverse effects associated with the therapy were caused by the toxicity of gemcitabine; thus, the safety and feasibility of this therapy were shown. The specific immune responses observed in patients with pancreatic cancer showing a good clinical response might be used as biomarkers to predict the efficacy of therapy. Five university hospitals, including The Jikei University, have collaboratively started a phase II clinical study of combination therapy with gemcitabine and a WT1 peptide vaccine to rapidly evaluate its therapeutic efficacy.

##### *A clinical study of a dendritic/tumor fusion cell vaccine to prevent the recurrence of glioblastoma on postoperative status*

Glioblastoma is a highly malignant brain tumor with an extremely poor prognosis. We

have reported that treatment with a dendritic/tumor fusion cell vaccine elicited a good response against glioblastoma. Although temozolomide has contributed to the improved prognosis of patients with glioblastoma, outcomes remain unsatisfactory. To prevent the recurrence of glioblastoma, we have provided postoperative combination treatment with fusion cell vaccine and temozolomide to patients with glioblastoma. This treatment has resulted in many cases of long-term survival without recurrence; 1 patient has survived more than 5 years. These results suggest that combination treatment with a fusion cell vaccine and temozolomide is superior to treatment with temozolomide alone for preventing the recurrence of glioblastoma. The mechanism of the possible synergistic effect of the fusion cell vaccine and temozolomide is now being investigated in in-vivo and in-vitro studies.

*Generation of a novel cancer vaccine composed of artificially constructed protein*

Using motif programming technology, we have constructed an artificial protein library by combining 4 peptide motifs associated with the MHC class I and class II epitopes of ovalbumin, an alpha-helical motif, and a randomized peptide sequence. Immunization with 2 artificial proteins, F37A and F182A, potentially induced ovalbumin-specific cellular immunity in mice. Subcutaneous injection with the artificial proteins efficiently presented the ovalbumin-specific MHC class I epitope through the cross-presentation pathway in antigen-presenting cells. These results demonstrate that vaccination with the motif-programmed artificial proteins of tumor antigen might be able to induce cellular immunity to elicit antitumor activity.

*Exploitation on novel tumor antigens by mass spectrometric analysis*

We have searched for candidate peptides for a novel cancer vaccine using the technology of proteomics. Peptides presented on HLA class I molecules are recognized by cytotoxic T lymphocytes with specific T-cell receptors. Some candidate peptides were identified among HLA-binding peptides from human prostate cancer cells by means of mass spectrometric analysis. Cancer specificity and the potential to become a target antigen for antitumor immunity are now being evaluated. One candidate peptide from an unexploited protein showed high messenger RNA expression in some human cancer cell lines but extremely low expression in many noncancerous tissues. Promising novel cancer vaccines might be generated on the basis of the structures of HLA class I-binding peptides of tumor cells.

*Cancer vaccine targeting tumor vessels generated from iPS cells*

Vaccination of mice with dendritic cells pulsed with lysate of induced vascular progenitor (iVP) cells derived from iPS cells showed potent antitumor activity against tumor-cell challenge, although vaccination with undifferentiated iPS cells did not. Suppression of tumor vasculature was observed in tumors formed in iVP-vaccinated mice. Furthermore, CD8<sup>+</sup> T cells from iVP-vaccinated mice showed significant cytotoxic activity against endothelial cells in vitro, but those from iPS-vaccinated mice did not. These results suggest that the immune response to iVP cells might suppress the development of tumor vessels, possibly by attacking endothelial cells in tumor tissue. The target antigen

responsible for this immune response is being identified with microarray analysis focusing on genes expressed in common in up-regulated genes of iVP cells through differentiation and constitutively expressed genes in tumor endothelial cells.

#### *Detection of antigenic peptides of tumor antigens from formalin-fixed tumor tissue*

Presentation of antigenic peptides of tumor antigens of MHC class I molecules on the surfaces of tumor cells is essential for antitumor immune responses. Although tumor antigen expression in tumor cells has been proven with immunohistochemical analysis, tumor cells with impaired antigen-processing capacity cannot express antigenic peptides despite high expression of tumor antigens. Conversely, expression of an antigenic peptide might be sufficient for an immune response, despite negative staining for tumor antigens on immunohistochemical analysis, if antigen processing is extremely rapid. Accordingly, direct demonstration of antigenic peptides of tumor antigens in tumor tissue is important for predicting the immune response to a tumor site. For this purpose, detection of antigenic peptides of known tumor antigens in formalin-fixed tumor tissue by means of liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis has been studied. We have previously achieved the quantitative detection of WT1 antigenic peptide from fresh pancreatic cancer tissue. The optimal conditions for detecting antigenic peptides of tumor antigens in formalin-fixed tumor tissue with LC/MS/MS are now being investigated. The quantity of antigenic peptides in tumor tissues might become a future predictive marker of the efficacy of cancer vaccine therapy.

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## Institute of DNA Medicine

### Department of Molecular Genetics

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Hisashi Yamada, *Professor and Director*

Takeshi Kawano, *Assistant Professor*

#### General Summary

Development, growth, and aging in humans are tightly controlled by genetics and epigenetics. The pathogenesis of many diseases is also thought to be a result of either a mutation of genes or a dysregulation of epigenetics. On the basis of this knowledge, clinical medicine is markedly changing. Further understanding of molecular pathogenesis will lead to more sophisticated treatment strategies. The diseases we are focusing on are hematological malignancies and pediatric cancers. We are also investigating spinal muscular atrophy (SMA), Alzheimer's disease, and retinal diseases. Molecular pharmacological studies of anticancer agents are another part of our research.

#### Research Activities

##### *Exploring hematological and pediatric malignancies*

Resistance of cancers to chemotherapy is explained with the cancer stem cell theory, which assumes that cancer stem cells are at the top of a cancer hierarchy. According to the classical understanding of this theory, cancer stem cells are never replaced by cells lower within the hierarchy. However, our data raise a question about this point. Cancer cells may change their phenotypes because of factors of their growth environment, including exposure to chemotherapeutic drugs. From this point of view, we are studying chemoresistance as a result of the plasticity of cancer cells. Eliminating cancer stem cells is required for successful treatment. However, the plasticity of cancer cells may change their vulnerabilities. We are studying this plasticity with JAS-R megakaryocytic leukemia cells. So-called cancer stem cells may consist of variable cells that change their characteristics and chemoresistance according to their growth conditions.

##### *Molecular pharmacology of anticancer agents*

Comprehensive cancer treatment often includes radiation therapy and chemotherapy. In our laboratory, we are investigating the anticancer activity of the following chemicals: telomerase inhibitors, histone deacetylase inhibitors, tyrosine kinase inhibitors, and DNA topoisomerase I inhibitors. We have found that these agents are suitable drugs for combination treatment. In particular, drugs that modulate epigenetic regulation may be ideal basal medicines, because many cancers have mutations of genes that regulate epigenetic control. Moreover, these drugs will reduce the risk of therapy-related malignancies, because they do not directly attack genomic DNA.

##### *Molecular genetic approach to neurological diseases*

SMA is degenerative disorder leading to muscular atrophy. Mutation of the survival

motor neuron 1 (SMN1) gene is responsible for the onset of SMA. Unlike other mammals, humans also have SMN2, a member of the same family as SMN1. Why intact SMN2 cannot compensate for the function of SMN1 in patients with SMA remains unclear. Our study found that RNA-binding proteins heterogeneous nuclear ribonucleoprotein A1 and A2 are involved in this obstruction through the splicing and translation of SMN2. These findings may contribute to new treatments for SMA.

Alzheimer's disease is an incurable degenerative disease that ultimately leads to dementia. It is occasionally difficult to predict the individual disease progression at the time of disease onset. We are investigating the relationship between the clinical characteristics of Alzheimer's disease and single-nucleotide polymorphisms of brain-derived neurotrophic factor and nerve growth factor. We believe that some of these single-nucleotide polymorphisms are useful for predicting disease progression.

## Publications

**Yamada O<sup>1</sup>, Ozaki K, Furukawa T<sup>1</sup>, Machida M<sup>1</sup>, Wang YH<sup>1</sup>, Motoji T<sup>1</sup>, Mitsuishi T<sup>1</sup>, Akiyama M, Yamada H, Kawauchi K<sup>1</sup>, Matsuoka R<sup>1</sup> (Tokyo Women's Med Coll).** Activation of STAT5 confers imatinib resistance on leukemic cells through the transcription of TERT and MDR1. *Cell Signal*. 2011; **23**: 1119-27.

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## Institute of DNA Medicine

### Department of Molecular Immunology

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Saburo Saito, Associate Professor and Director  
Nobutake Akiyama, Assistant Professor

Daitaro Kurosaka, Associate 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

##### *Pleiotropic function of interleukin-31*

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. To investigate the function of IL-31, IL-31 transgenic mice were created in our laboratory. In addition to scratching behavior and hair loss as reported previously, enhancement of the serum immunoglobulin (Ig) E level was observed in the IL-31 transgenic mice. Moreover, these pleiotropic functions were verified by the administration of IL-31 into normal mice. To further analyze the mechanisms of IgE production by IL-31, we are seeking factors enhancing T helper type 2 (Th2) cytokine production, focusing on the IL-31 receptor-expressing cells, such as keratinocytes, macrophages, and granulocytes. We found that activated M2 macrophages become target cells in the presence of IL-31 to promote Th2 cell differentiation.

Furthermore, to investigate the function and the locations of IL-31 or the IL-31 receptor, 2 strains of IL-31 or IL-31 receptor (IL-31R) knockout/*LacZ* knockin mice were generated. In *IL-31R*<sup>+/lacZ</sup> knockin mice 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside staining was limited to the hair matrix. To produce offspring with a genetic identity for the analysis of the pleiotropic functions of IL-31, the heterozygous mouse will be backcrossed 6 or more times into the C57B/6J genetic background.

##### *A rice-based edible vaccine expressing Japanese cedar pollen allergens induces oral tolerance in Japanese monkeys with Japanese cedar pollinosis*

Japanese cedar (*Cryptomeria japonica*: CJ) pollinosis affects more than 30% of the Japanese population and is, thus, one of the most common diseases in Japan. Furthermore, CJ pollinosis has been found to occur naturally in Japanese monkeys (*Macaca fuscata*), which show symptoms similar to those of human patients.

Plants have recently been recognized as a form of bioreactor for the cost-effective production of large-scale recombinant proteins. The edible tissue of plants further provide the significant benefit of being a simple method of mucosal delivery of vaccines without the need for complicated purification steps.

Our previous study showed that oral administration of transgenic rice seeds that have accumulated high concentrations of polypeptides derived from CJ pollen allergens to mice reduces their serum IgE levels and T-cell proliferative responses to CJ allergens, proving the efficacy of oral immunotherapy for the treatment of pollinosis.

In this study, the transgenic rice plants that had accumulated high concentrations of JC allergens were used for oral immunotherapy for CJ pollinosis in monkeys. Five monkeys with CJ pollinosis were fed once a day with 20 g of the rice seeds containing about 50 to 60 mg of allergens for 3 months. No side effects, such as urticaria, dyspnea, vomiting, and weight loss, were observed during immunotherapy. One and a half months after the start of feeding, proliferative responses of T cells to JC allergens in 4 of 5 monkeys were significantly inhibited compared with those in monkeys at the start of feeding. However, their T-cell responses to CJ allergens were restored 1 month after the end of feeding.

On the other hand, in healthy monkeys without CJ pollinosis, the side effects and the induction of immune responses to CJ allergens were not observed after oral administration of transgenic rice seeds.

These results indicate that oral immunotherapy with transgenic rice seeds is a safe and effective treatment for pollinosis.

#### *Construction of a new anticancer strategy focused on glycosylation*

We are developing a novel anticancer strategy that induces cytotoxic T cells against non-polarized cells represented by cancer cells, by enhancing MHC class I-restricted antigen presentation by inhibiting N-glycosylation.

Analysis of the N-glycosylation structure that controls the secretion of IL-31 showed that some structures of N-glycosylation were able to enhance MHC class I-restricted antigen presentation. On the basis of this finding, we are developing a new vaccine that induces cytotoxic T cells against cancer or viruses with artificial immature N-glycosylated proteins.

#### **Publications**

**Watanabe M, Fujioka K, Akiyama N, Takeyama H, Manabe N, Yamamoto K, Manome Y.** Conjugation of quantum dots and JT95 IgM monoclonal antibody for thyroid carcinoma without abolishing the specificity and activity of the antibody. *IEEE Trans Nanobioscience*. 2011; **10**: 30-5.

**Ikura K, Katsunuma T, Saika S, Saito S, Ichinohe S, Ida H, Saito H, Matsumoto K.** Peripheral blood mononuclear cells from patients with bronchial asthma show impaired innate immune responses to rhinovirus in vitro. *Int Arch Allergy Immunol*. 2011; **155** suppl 1: 27-33.

## Institute of DNA Medicine

### Department of Molecular Cell Biology

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Yoshinobu Manome, *Professor*

#### General Summary

The goal of our department is to perform medical science research based on the molecular biology of cells. For this reason, molecular events of cells under physiological and pathological conditions are analyzed. To achieve our goal, both morphological and biochemical approaches are applied, and methods for modifying the transcription and expression of nucleic acids are used. The methods include transfection of DNA or short interfering RNA to modulate gene expression. Also, to quantify target molecules, we use such methods as labeling with fluorescent nanoparticles, conjugation to sensors, and amplification with radiolabelled materials. By introducing the methods of molecular and cellular biology, we are addressing clinical problems.

#### Research Activities

##### *Development of a nucleic acid delivery system for malignant glioma cells by acoustic energy*

Malignant glioma is an intractable disease. Many adjuvant therapies, such as radiotherapy, chemotherapy, and immunotherapy, have been developed. Nevertheless, the prognoses of patients remain unsatisfactory. For this reason, we are exploring alternative strategies, such as sonodynamic therapy. Despite the poor prognosis of patients with malignant glioma, metastasis outside the central nervous system is rare, and the cause of death in most cases is local recurrence. Therefore, if an effective local therapy were established, patients would live longer, and even complete cure could be expected. Against this background, we have developed a theragnosis system, which is a combination of therapy and diagnosis, for glioma. With this system, ultrasound is applied to local glioma lesions for both diagnosis and treatment. In addition, we are developing a nucleic acid delivery system based on the theragnosis system. We found that down-regulation of Rho-associated kinase 2 (ROCK2) by short hairpin RNA inhibited tumor growth *in vitro* and increased sensitivity to the antineoplastic agent temozolomide. Also, forced expression of phosphatase and tensin homologue (PTEN) demonstrated the same effects. Both methods prolonged the G2 phase of the cell cycle and increased sensitivity to alkylating agents. While these molecules are key targets for therapy, other candidates and other types of malignancy are being screened.

##### *The antioxidative actions of urocortin I on cardiac myocytes*

Oxidative stress is a major pathological factor in heart disease. Recently, many protective cardiovascular agents, such as atrial/brain natriuretic peptides, are used to treat heart disease. The protein urocortin I exerts several beneficial effects involved in cytoprotec-

tion, including the antioxidative action. The antioxidative action of urocortin I, however, has not been thoroughly investigated. Therefore, the antioxidative action of urocortin I in HL-1 cardiomyocytes induced by cardiovascular pathological agents was investigated with nicotine. We found that urocortin I, not urocortin II, attenuated nicotine-induced oxidative stress. The mechanism of the antioxidative action of urocortin I is also being investigated.

#### *Development of a high-accuracy, high-sensitivity, and rapid diagnosis system for thyroid carcinoma*

We developed biomedical applications for histochemistry and cytochemistry using a biotinylated JT-95 monoclonal antibody that recognizes an antigen of thyroid carcinomas. Moreover, we optimized the enzyme-linked immunosorbent assay system for blood tests with both JT-95 and biotinylated JT-95. To support the clinical use of these applications, we have been studying the accuracy of the detection system.

#### **Publications**

**Takeyama H, Kyoda S, Okamoto T, Manome Y, Watanabe M, Kinoshita S, Uchida K, Sakamoto A, Morikawa T.** The expression of sialic fibronectin correlates with lymph node metastasis of thyroid malignant neoplasmas. *Anticancer Res.* 2011; **31**: 1395-8.

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**Ikeda K, Saito T, Tojo K.** Efonidipine, a Ca<sup>2+</sup>-channel blocker, enhances the production of dehydroepiandrosterone sulfate in NCI-H295R human adrenocortical carcinoma cell. *Tohoku J Exp Med.* 2011; **224**: 263-71.

## Institute of DNA Medicine

### Project Laboratory for Kidney Regeneration

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Takashi Yokoo, *Assistant Professor and Director*

#### General Summary

Recently, pluripotent stem cells have been successfully isolated and established from various tissues, which has brought the possibility of using somatic stem cells for organ regeneration one step closer to realization. For stem cell to be used clinically, they must be differentiated into functionally mature forms, and many researchers have attempted to establish individual somatic cell types. However, only very few cell types, such as pancreatic beta cells and cardiac myocytes, have been successfully established thus far. The major reason for this lack of success is that the developmental programs that cause stem cells to differentiate into mature cells consist of numerous factors, some of which are as yet unknown, and contribute to each other temporally and spatially in a tissue-specific manner. Clarifying the developmental program for each cell type one by one is extremely challenging. In this context, we have previously found that bone marrow-derived mesenchymal stem cells (MSCs), but not embryonic stem (ES) cells or induced pluripotent stem (iPS) cells, integrate into the kidney structure and acquire some renal functions when cultured with an immature metanephros *in vitro*. Fetal organs, such as the metanephros, have been suggested to be less immunogenic and more useful for transplantation because: 1) antigen-presenting cells that mediate direct host recognition of alloantigens and xenoantigens would be absent; 2) donor antigens, such as MHC class I and II antigens, might not be expressed by developing organs; and 3) the immune response to transplanted fetal tissue differs from that to adult tissue in terms of eliciting a helper T type 2 cell-biased response when the target organ is of fetal origin. In fact, direct comparison of xenotransplantation clearly shows the immune advantage of developing precursor transplants over developed adult transplants in fully immunocompetent hosts. On the basis of these observations, we speculated that we could use the developmental program of a developing organ by transplanting it into an ectopic site where it continued development *in vivo*. This procedure would facilitate the inward migration of autologous stem cells, which would then be stimulated by the developmental program of the xeno-organ to mature into tissue-specific cells.

In addition, conventional xenotransplantation should require continuous and strong immunosuppression to avoid any humoral rejection that occurs across the xenogeneic barrier, which evokes various adverse effects, including carcinogenicity and severe infection. In contrast, this *in vivo* programming system temporarily uses xeno-organs as the source of the developmental program, and after the tissue of interest has been established, the xenocomponent is no longer needed and can be discarded. Therefore, we introduced a cell fate-regulating system, in which a suicide gene is expressed on demand, and combined this system with the *in vivo* programming system.

## Research Activities

This year, we established a xenotransplantation model in which the differentiation of endogenous MSCs into mature erythropoietin-producing tissue is controlled in a niche provided by a developing xenometanephros. Transplantation of rat metanephroi into mouse omentum, and similarly that of pig metanephroi into cat omentum, led to the recruitment of host cells and to erythropoietin production. Erythropoietin-expressing cells were not differentiated from integrating vessels because they did not co-express endothelial markers (Tie-2 and vascular endothelial cadherin). Instead, erythropoietin-expressing cells were shown to be derived from circulating host cells, as indicated by enhanced green fluorescent protein (EGFP) expression in the grown transplants of chimeric mice bearing bone marrow from a transgenic mouse expressing EGFP under the control of the erythropoietin promoter. These results suggest that the recruitment and differentiation of donor cells in a developing xenotransplanted organ is consistent between species. The cells responsible for erythropoietin expression were identified as MSCs by injecting human bone marrow-derived MSCs and endothelial progenitor cells into non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice. Furthermore, using metanephroi from transgenic ER/E2F1 suicide-inducible mice, the xenotissue component could be eliminated, leaving autologous erythropoietin-producing tissue. Our findings might help alleviate adverse effects due to long-term immunosuppression and help mitigate ethical concerns.

## Reviews and Books

**Yokoo T, Matsumoto K, Yokote S.** Potential use of stem cells for kidney regeneration. *Int J*

*Nephrol.* 2011; **2011**: 591731.

## Department of Neuroscience

### Division of Neuropathology

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Satoshi Kurihara, *Professor and Director*  
Junko Fujigasaki, *Assistant Professor*

Takahiro Fukuda, *Assistant Professor*

#### General Summary

Our research projects have concerned neurodegenerative disorders caused by 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

##### *Pathophysiological study of neuronal organelles in lysosomal diseases*

**Objective:** The aim of this study was to investigate the pathophysiology of neuronal organelles in lysosomal disorders.

**Material and methods:** We analyzed the central nervous system (CNS) of Niemann-Pick disease type C and prosaposin deficiency model mice by means immunohistochemical studies with antibodies against early endosome antigen 1 (endosomes), trans-Golgi network 38 (Golgi apparatus), cytochrome c oxidase subunit IV (mitochondria), calnexin (endoplasmic reticulum), S6 ribosomal protein (ribosomes), lysosome-associated membrane protein 2 (lysosomes), and catalase (peroxisomes).

**Results:** In the CNS neurons of Niemann-Pick disease type C and prosaposin deficiency mice, swollen lysosomes accumulated. Structurally preserved peroxisomes and Golgi apparatuses decreased slightly in number. Mitochondria, endosomes, endoplasmic reticulum, and ribosomes decreased markedly in number.

**Discussion:** After being sorted in endosomes, most proteins are rapidly recycled. Degraded proteins are packaged into lysosomes and then processed into the ubiquitin-proteasome system or the autophagy-lysosome system. In lysosomal storage diseases, recycled endosomes are inhibited, and degraded proteins accumulate in lysosomes. Mitochondria depletion leads to an energy crisis and decreases in the synthesis activity of proteins and lipids in ribosomes and endoplasmic reticulum.

##### *A case of chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids syndrome diagnosed with brain biopsy*

A 28-year-old woman consulted our hospital due to ataxia, diplopia, and facial paresthesia. Magnetic resonance disclosed patchy spotlike gadolinium enhancement with a “salt-and-pepper”-like appearance in the cerebellum and pons. Brain biopsy was performed before corticosteroids were administered. The biopsy specimens showed a scattered and perivascular infiltration of small, mature lymphocytes in the white matter of the cerebellum.

The lymphocytes were predominantly T cells admixed with CD4-positive and CD8-positive cells. Cells immunoreactive for Olig2 with slightly enlarged nuclei appeared; however, an inflammatory reaction rather than a neoplastic lesion was suspected. After biopsy, treatment with corticosteroids lead to symptomatic improvement and the reduction of lesions on magnetic resonance. The pathological findings and the clinical course led to a diagnosis of chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids (CLIPPERS) syndrome. In 2010, this syndrome was identified as a rare chronic inflammatory CNS disorder responsive to immunosuppressive therapy. The diagnosis in this case required careful assessment of the clinical course and pathological findings.

## Publications

**Kawagoe S, Higuchi T, Meng XL, Shimada Y, Shimizu H, Hirayama R, Fukuda T, Chang H, Nakahata T, Fukada S, Ida H, Kobayashi H, Ohashi T, Eto Y.** Generation of induced pluripotent stem (iPS) cells derived from a murine model of Pompe disease and differentiation of Pompe-iPS cells into skeletal muscle cells. *Mol Genet Metab.* 2011; **104**: 123-8.

**Wang Z, Fukuda T, Azuma T, Furuhashi H.**

Safety of low-frequency transcranial ultrasound in permanent middle cerebral artery occlusion in spontaneously hypertensive rats. *Cerebrovasc Dis.* 2012; **33**: 23-9.

**Inagaki T, Fukuda T, Ohta A, Hano H.** No oncogenic role of WT1 in the peripheral nerve sheath tumors. *Jikeikai Med J.* 2011; **58**: 95-102.

## Department of Neuroscience Laboratory of Neurophysiology

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Fusao Kato, *Professor and Director*

Ayako M. Watabe, *Assistant 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 optimise 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  $\text{Ca}^{2+}$  concentration, and optogenetic approaches to activate a specific set of neurons by light, in living brain tissues from normal animals, animal models of various types of disease, 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 a rat model of chronic neuropathic pain, we demonstrated that 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 the principal role in expression of emotional behaviors, involves structural consolidation. We also demonstrated that in the streptozocin-induced model of painful diabetes neuropathy, the synaptic potentiation in the amygdala is established selectively in the parabrachial-capsular synapses, unlike in other models. This finding further confirms the notion that the mechanism of synaptic potentiation in the central amygdala, which underlies the enhanced link between nociception and negative emotions, depends largely on the modality and duration of chronic pain.

#### *Synaptic mechanism underlying acquisition and extinction of fear memory*

The Pavlovian fear-conditioning paradigm depends on the association between a contiguously applied cue (e.g., tone) and an aversive signal (e.g., electric shock). We aimed to establish transgenic mice that express specific fluorescent marker proteins in response to fear conditioning or its extinction to enable selective fluorescence-guided recording of the identified amygdala neurons in brain slices after behavioral tests. This preparation will enable analyses of specific synaptic changes in the neurons involved in these processes.

### *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. We found that lactate transport is essential for maintaining the postsynaptic responses both in the presence and the absence of glucose supply.

### *Specific mechanism underlying motor neuron vulnerability*

We have already demonstrated that, in the hypoglossal motor neurons, anoxia and hypoxia facilitate glycine release in an action potential-independent manner. We found that this facilitation of glycine release also occurs in facial motor neurons but not in oculomotor neurons, in which anoxia facilitates GABA release. This difference in the anoxia responses of the inhibitory transmission between distinct motor neurons might provide a basis for the distinct vulnerability of these motor neurons in motor neuron degenerative diseases.

## Publications

**Aoyama R, Okada Y, Yokota S, Yasui Y, Fukuda K, Shinozaki Y, Yoshida H, Nakamura M, Chiba K, Yasui Y, Kato F, Toyama Y.** Spatiotemporal and anatomical analyses of P2X receptor-mediated neuronal and glial processing of sensory signals in the rat dorsal horn. *Pain*. 2011; **152**: 2085–9.

**Yasui Y, Masaki E, Kato F.** Esmolol modulates inhibitory neurotransmission in the substantia gelatinosa of the spinal trigeminal nucleus of the rat. *BMC Anesthesiol*. 2011; **11**: 15.

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**Nomoto M<sup>1</sup>, Takeda Y<sup>1</sup>, Uchida S<sup>1</sup>, Mitsuda K<sup>1</sup>, Enomoto H<sup>1</sup>, Saito K<sup>1</sup>, Choi T<sup>1</sup>, Watabe AM, Kobayashi S<sup>2</sup>, Masushige S<sup>1</sup>, Manabe T<sup>2</sup>, Kida**

**S<sup>1</sup> (<sup>1</sup>Tokyo Univ Agriculture, <sup>2</sup>Univ Tokyo).** Dysfunction of the RAR/RXR signaling pathway in the forebrain impairs hippocampal memory and synaptic plasticity. *Mol Brain*. 2012; **5**: 8.

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## Institute for High Dimensional Medical Imaging

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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., three-dimensional (3D) and four-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 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 emergency safety mechanism, improved the user interface, and carried out basic evaluations of the degree of accuracy and the delay in the robot's movement to bring the system closer to clinical application. In addition to pursuing our main research project, we started developing a camera for surgery navigation and various other surgical apparatuses.

#### *Development of a simulator for the endoscopic surgical robot system*

To perform surgery with the surgical robot system described above, the operator requires training because the operative method differs greatly from that of conventional surgery. Therefore, we are developing a simulator system for animal experiments that has the same functions as the actual surgical robot system.

This year, we improved the modeling and texture of the organ model in the surgical field so that operators can train in an environment as close as possible to the actual environment. In addition, we trained numerous people in the use of the system. The purpose of this training was to accumulate data about the time needed to complete tasks, the amount of blood loss, and the changes in robot-arm trajectory during training to verify the effectiveness of training with the system.

#### *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 we performed 7 navigation operations in collaboration with the Department of Surgery and 5 in collaboration with the Department of Otorhinolaryngology in a high-tech navigation operating room in the Daisan Hospital. In joint research with the Department of Surgery, by adopting short-axis 3D laparoscope, we were able to acquire a stereoscopic view of the resection plane and target regions while navigating. In sinus surgery performed with the Department of Otorhinolaryngology, we were able to navigate through an oblique-viewing endoscope as well as through a forward-viewing 3D endoscope. In this way, we were able to provide navigation information for the entire operation.

#### *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.

This year, we have analyzed the position, depth, and angle of a victim's wounds in 3D using the X-ray CT data set from a case of attempted murder.

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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**Hattori A, Suzuki N, Ieiri S, Tomikawa M,**

**Kenmotsu H<sup>1</sup>, Hashizume M<sup>1</sup> (Kyushu Univ).** Training system for NOTES and SPS surgery robot that enables spatiotemporal retrospective analysis of the training process. *Stud Health Technol Inform.* 2012; **173**: 166-70.

## Institute of Clinical Medicine and Research

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Norio Tada, *Professor and Director*

Akihito Tsubota, *Associate Professor*

Sadayori Hoshina, *Associate Professor and Deputy Director*

Yoshihisa Namiki, *Assistant Professor*

### General Summary

The aim of our research is to bridge 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 separate radioactive compounds by means of magnetic basket-shaped nanocapsules containing decontaminants. We also made progress in gene technology, especially in the treatment of hepatitis C virus (HCV) infection and liver cancer. Two of our major research topics are the transporter of ribavirin into hepatocytes and the function of microRNA/messenger (m) RNA. In the field of lipid metabolism related to atherosclerosis, we have reassessed lipoprotein cholesterol separated with our newly developed ion-exchange chromatography; last year we used this chromatography method to measure lipoprotein (a), an atherosclerotic lipoprotein, with a special apolipoprotein called apolipoprotein (a).

### Research Activities

#### *Transporter gene in the treatment of chronic hepatitis C virus infection*

Combination therapy with pegylated interferon and ribavirin is the standard-of-care treatment for chronic infection with hepatitis C virus (HCV). In this treatment, exposure of HCV to ribavirin in hepatocytes is critical for virus eradication. Ribavirin is transported into hepatocytes by cell-membrane transporters. We are investigating the function of the transporters and the association of single nucleotide polymorphisms of the gene with treatment response.

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

We are profiling and analyzing the expression of microRNA/messenger (m) RNA in the liver tissue of patients with chronic HCV infection who would receive standard-of-care treatment. We are analyzing whether the microRNA/mRNA candidates can be associated with treatment response in chronic HCV infection. When the candidates affect the treatment outcome, the function of the microRNA/mRNA will be investigated in detail.

#### *The temporal and spatial manipulation of "basket-type organic/inorganic-hybrid structure" as a future theragnostic nanomedicine*

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 and perfectly timed” diagnosis and treatment.

We aim to realize innovative nanomedicine in which we can remotely control the accumulation, release, and effects of drugs with nanocapsules 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 are 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 and biotechnology.

#### *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 hyperlipidemia 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

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Hiroataka James Okano, *Professor*

### General Summary

Regenerative medicine is rapidly moving toward translation to clinical medicine. However, a better understanding of the molecular pathways leading to human diseases is required for regenerative medicine to succeed. Good animal models will play a key role in research to increase our understanding of the diseases. Disease models in genetically engineered mice are extremely useful but do not always precisely recapitulate the pathophysiology of human disease, especially disorders of the central nervous system. We have recently attempted to create a transgenic primate model of human neurodegenerative diseases through forced expression of dominant mutant genes. On the other hand, induced pluripotent stem (iPS) cell technology has given us the ability to generate and expand various types of differentiated cell from patient-derived cells; iPS cells are now being applied to cell therapy and being used to study of the mechanisms of disease. Advances in disease modeling with patient-derived cells and nonhuman primates will have an enormous affect on future opportunities and advances in biomedical research.

### Research Activities

#### *Therapeutic strategies for the damaged central nervous system*

Recent advances in developmental and stem-cell biology have made regeneration-based therapies feasible for patients with damaged central nervous systems, including those with spinal cord injuries, Parkinson's disease, or stroke. Understanding and then controlling the appropriate regulatory mechanisms in neural stem cells (NSCs) will be important milestones in the development of regeneration-based treatments for damaged central nervous system tissue. Previously, we observed how transplanted iPS cell-derived NSCs were integrated in the injured spinal cord and differentiated into various kinds of cell, including neurons, astrocytes, and oligodendrocytes. Although the precise mechanism of symptomatic improvement remains unclear, NSC transplantation has promoted functional recovery in experimental studies in rats and nonhuman primates.

#### *In-vivo imaging technology applied to regenerative medicine*

Bioluminescence imaging is an efficient and powerful method for longitudinal comparison of cell survival and migration. Cell therapies can be more quickly optimized and refined with imaging, which is widely applicable to various types of regenerative medicine, including stem-cell therapies. We used *in-vivo* bioluminescence imaging to noninvasively assess the survival and residence time of transplanted NSCs at injury sites in living animals. Photon signals from these cells were detectable through normal tissues, such as bone and skin, with ultrasensitive cooled charged-coupled device cameras for 10

months or more after transplantation into the injured spinal cords of mice.

### *Common marmoset as a primate disease model*

The common marmoset (*Callithrix jacchus*) is becoming an increasingly popular primate animal model in biomedical research, because of its advantage of translation to genetically similar human systems. Marmosets, because of their small size and high reproductive rate, are suitable subjects for transgenic modification. Recently, transgenic marmosets were successfully created by gene transduction into embryos; this animal model is genetically similar to humans and can be used to study human neurodegenerative diseases, such as Parkinson's disease and amyotrophic lateral sclerosis. Good animal models will play a key role in research to increase our understanding of the pathophysiology of neurodegenerative diseases. We have recently attempted to create a transgenic marmoset model of human neurodegenerative diseases through forced expression of dominant mutant genes.

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## Medical Engineering Laboratory

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Masayuki Yokoyama, *Associate Professor and Director*

Hiroshi Furuhashi, *Professor*

### General Summary

Medical engineering is an essential foundation for developments in medicine. In our laboratory, there are 2 key technologies; ultrasound and polymeric biomaterials. We have developed ultrasound technology for a new thrombolytic treatment for acute ischemic stroke. Our ultrasound research is characterized by the use of medium-frequency ultrasound and close collaborations with clinical departments and basic science departments, both in our university and hospital and others'. For the other key technology, polymeric biomaterials, we have applied these materials mainly for drug delivery systems. Recently, we have also applied polymeric materials to imaging diagnosis through the synthesis of new polymeric contrast agents. In particular, we study polymeric micelle systems that can deliver both drugs and contrast agents. Therefore, these systems are called "theranostic" systems because of the dual functions of therapy and diagnostics. Additionally, some of our research studies combine ultrasound and polymeric biomaterials. One example is ultrasound-assisted targeting of polymeric drug carrier systems.

### Research Activities

#### *Medical application of ultrasound*

We have applied ultrasound for a transcranial therapy against brain ischemic stroke. Against this serious disease, injection of tissue plasminogen activator (t-PA) has been the only effective therapy. However, significant increases in therapeutic effects are strongly wanted. Transcranial ultrasound can enhance the thrombolytic activity of t-PA. Our technology features the use of medium-frequency ultrasound, which is known to enhance thrombolysis more than does the ordinal ultrasound. However, the medium-frequency ultrasound is believed to be associated with a high risk of brain hemorrhage. To resolve the hemorrhage risk problem, we control both the irradiation period and the interval of irradiation; we have shown in models of hypertensive brain ischemia that, the ultrasound irradiation is both safe and effective. Furthermore, we have found that the modulation of ultrasound reduced unfavorable reflections of the applied ultrasound. We have been developing this new therapy through a "super special consortium for supporting the development of cutting-edge medical care" program supported by the Ministry of Health, Labour and Welfare, Japan.

#### *Polymeric micelle drug carrier systems*

Polymeric micelles are assemblies of synthetic polymers, and have been actively applied for drug targeting. Associate Professor Yokoyama, 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 science technology in the polymeric micelle systems. We are studying the immunological properties of polymeric micelles. Curiously, the polymeric micelle carriers are very different from liposome systems. Although both the surfaces of carrier systems possess poly(ethylene glycol), the polymeric micelle carriers induced no or low immunological responses of the accelerated blood clearance phenomenon. This low immunogenicity presents a great advantage of the polymeric micelle systems. We are also studying basic chemistry to analyze, through the use of the Super Photon Ring 8 Giga-electronvolt facility, the drug-incorporated inner core and to prepare polymeric micelles with cross-linked inner cores through photochemistry. These cross-linked micelles are extremely useful for determination of the in vivo fates of polymeric micelles.

#### *Polymer-based contrast agents for image diagnosis*

We have developed new polymeric micelle contrast agents for magnetic resonance imaging (MRI). These contrast agents were proven to be targeted at solid tumor sites and to exhibit clear magnetic resonance images of very small tumors. Therefore, the polymeric micelles can be used for the “theranostics” of tumors because the polymeric micelles can target both drugs and contrast agents to solid tumors. Furthermore, we are studying a novel application of the polymeric carrier system for diagnosing brain ischemic stroke. We observed that a polymeric micelle MRI contrast agent was successfully targeted to a specific site in the ischemic hemisphere and provided high-contrast images that were not obtained with a conventional low-molecular-weight MRI contrast agent. This high contrast was obtained in a short time such as 20 to 60 minutes after the contrast agent was injected intravenously. Therefore, the polymeric micelle carrier system may be extremely useful for both the diagnosis and the therapy of ischemic stroke.

#### **Publications**

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**Sawaguchi Y, Wang Z, Furuhashi H.** Ultrasound control of the growth of thrombus: potential for the embolus growth suppression & the reocclusion prevention (in Japanese). *Japanese Journal of Medical Ultrasonics.* 2011; **38**: 549-55.

**Furuhashi H.** Prospect of sonothrombolysis for the next generation of ultrasonic therapy from the present time to the near future (in Japanese). *Igaku no Ayumi.* 2011; **238**: 189-95.

**Furuhashi H.** Safety management of ultrasonic instruments for diagnosis (in Japanese). *Rinsho Gazo.* 2011; **27** 4 Suppl: 6-9.

**Yokoyama M.** Clinical applications of polymeric micelle carrier systems in chemotherapy and image diagnosis of solid tumors. *Journal of Experimental & Clinical Medicine.* 2011; **3**: 151-8.

## Division of Clinical Pharmacology and Therapeutics

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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. They 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 pilot study in 3 major hospitals, including our hospital, according to a case-cohort design in which detailed data were collected from all cases and in 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.

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

**Saito I<sup>1</sup>, Suzuki H<sup>2</sup>, Kageyama S, Saruta T<sup>1</sup>**  
(<sup>1</sup>Keio Univ, <sup>2</sup>Saitama Med Sch). Treatment of hypertension in patients 85 years of age or older: a J-BRAVE substudy. *Clin Exp Hypertens*. 2011;

33: 275-80.

**Kageyama S, Ueda S<sup>1</sup>, Mochizuki K<sup>2</sup>, Miyakawa M<sup>3</sup>, Sugawara M<sup>4</sup>, Nakayama M<sup>5</sup>, Ohashi Y<sup>6</sup>, Saito I<sup>7</sup>, Saruta T<sup>7</sup>; OCEAN Study**

**Group<sup>8</sup>** (<sup>1</sup>Ryukyu Univ, <sup>2</sup>Mochizuki Clin, <sup>3</sup>Miyakawa Clin, <sup>4</sup>Sugawara Clin, <sup>5</sup>Nakayama Clin, <sup>6</sup>Univ Tokyo, <sup>7</sup>Keio Univ, <sup>8</sup>Pub Health Res Found). Optimal Combination of Effective ANti-hypertensives (OCEAN) study: a prospective, randomized, open-label, blinded endpoint trial-ratio-nale, design and results of a pilot study in Japan. *Hypertens Res*. 2012; **35**: 221–7.

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**Watanabe H<sup>1</sup>, Kageyama S, Kusuoka H<sup>2</sup>, Ono S<sup>3</sup>, Saito K<sup>4</sup>, Isobe T<sup>5</sup>, Kakee N<sup>6</sup>, Kurihara C<sup>7</sup>, Sakuhiro T<sup>8</sup>, Aoki H<sup>8</sup>, Tsujide K<sup>8</sup>, Nabeoka Y<sup>9</sup>, Morishita N<sup>2</sup>, Suzuki C<sup>10</sup>, Kachi S<sup>1</sup>, Takehara K<sup>6</sup>, Tsujimoto Y<sup>11</sup>, Kondo E<sup>1</sup>, Komori Y<sup>1</sup>** (<sup>1</sup>Hamamatsu Univ, <sup>2</sup>Osaka Natl Hosp, <sup>3</sup>Univ Tokyo, <sup>4</sup>Pharm Med Dev Agcy, <sup>5</sup>Keio Law Sch, <sup>6</sup>Natl Ctr Child Health Dev, <sup>7</sup>Natl Inst Radiol Sci, <sup>8</sup>Jpn Pharm Manu Assoc, <sup>9</sup>Chugai Pharm Co., Ltd., <sup>10</sup>Seirei Hamamatsu Gen Hosp, <sup>11</sup>Cons Organiz Med Law). Protection of human subjects and compensation for research-related injuries: Proposal of explanation sheet based on survey of the actual status in Japan (in Japanese). *Rinsho Hyoka*. 2011; **39**: 5–29.

## Division of Molecular Epidemiology

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Mitsuyoshi Urashima, Associate 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*

From May 2011 through March 2012, we held 10 seminars' about strategies for clinical studies for healthcare practitioners at The Jikei University. In 2011, 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 in Global Health

### Publications

**Kakuda W, Abo M, Shimizu M, Sasanuma J, Okamoto T, Yokoi A, Taguchi K, Mitani S, Harashima H, Urushidani N, Urashima M; NEURO Investigators.** A multi-center study on low-frequency rTMS combined with intensive occupational therapy for upper limb hemiparesis in post-stroke patients. *J Neuroeng Rehabil.* 2012; **9**: 4.

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**Yokote K, Shimano H, Urashima M, Teramoto T.** Efficacy and safety of pitavastatin in Japanese patients with hypercholesterolemia: LIVES study and subanalysis. *Expert Rev Cardiovasc Ther.* 2011; **9**: 555-62.

**Kaise M, Miwa J, Tashiro J, Ohmoto Y, Morimoto S, Kato M, Urashima M, Ikegami M, Tajiri H.** The combination of serum trefoil factor 3 and pepsinogen testing is a valid non-endo-

scopic biomarker for predicting the presence of gastric cancer: a new marker for gastric cancer risk. *J Gastroenterol.* 2011; **46**: 736-45.

**Akiyama N, Segawa T, Ida H, Mezawa H, Noya M, Tamez S, Urashima M.** Bimodal effects of obesity ratio on disease duration of respiratory syncytial virus infection in children. *Allergol Int.* 2011; **60**: 305-8.

## Division of Clinical Epidemiology

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Masato Matsushima, *Associate Professor and Director*

### General Summary

The Division of Clinical Epidemiology was founded in 2009 as one part of the Research Center for Medical Science to promote the activity of clinical research, clinical epidemiology and education concerning them. Our aim is to support clinicians to solve their own problems in daily practice by epidemiological/clinical research skills.

The research agenda covered by our division are, moreover, medical communication, evaluation of medical care, behavioral medicine, outcome research, qualitative research as well as disease-oriented epidemiological research. Especially, we aim at making evidence in the field of primary-care due to the lack of evidence although primary-care is a front-line of practice.

As a contribution to the pregraduate education, our division has classes of “Evidence-based Clinical Practice (EBCP)” to make medical students skillful doctors able to employ evidence-based approach.

Our post-graduate education concentrates on the methodology of clinical/epidemiological research and biostatistics. “The educational program for primary-care on clinical research methodology”, which was started in 2007 by financial support of the Ministry of Health, Labour and Welfare, was renewed as “Jikei Clinical Research Program for Primary-care” in 2009. The aim of this program is to have a primary-care physician be a clinician-researcher.

### Research Activities

#### *Chronic care model*

Chronic care model was developed during 1990's in US to improve chronic illness care by refining care-provider system especially in a primary-care setting. The aim of this research is to clarify the usefulness of the chronic care model in Japan.

The plan consisted of 3 steps. The first was to make the official Japanese version of the assessment form “Assessment of Chronic Illness Care (ACIC)” by following WHO procedure, e.g., translation, back translation, and pilot study. The second step was to compare the quality of diabetes care between specialists in diabetes and primary-care physicians as non-specialists. The last step was to evaluate the validity of ACIC to examine the correlation between ACIC scores and the measurement of urinary albumin excretion.

#### *Patient Enablement Instrument*

The concept of “patient enablement” involves patients' perceptions of ability to understand and cope with illness. Patient Enablement Instrument (PEI) is a questionnaire developed in UK to evaluate patient enablement. The aim of this study was to develop PEI Japanese version and examine its validity and reliability. Two principal compo-

nents, coping and independence have been identified in Japanese patient enablement by PEI Japanese version with high validity and reliability.

#### *The cohort study in home medical care*

The cohort study in home medical care was planned to evaluate the incidence of death at home. This cohort will be comprised with patients receiving home medical care by regular visits of general practitioner. Financial support for this research was provided by the Ministry of Health, Labour and Welfare.

#### **Publications**

**Yokoyama H, Matsushima M, Kawai K, Hirao K, Oishi M, Sugimoto H, Takeda H, Minami M, Kobayashi M, Sone H; Japan Diabetes Clinical Data Management Study Group.** Low incidence of cardiovascular events in Japanese patients with type2 diabetes in primary care settings: a prospective cohort study. *Diabet Med.* 2011; **28**: 1221-8.

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**Date T, Yamane T, Yamashita S, Matsuo S, Matsushima M, Inada K, Taniguchi I, Yoshimura M.** Paradoxical clearance of natriuretic peptide between pulmonary and systemic circulation: a pulmonary mechanism of maintaining natriuretic peptide plasma concentration in obese individuals. *J Clin Endocrinol Metab.* 2012; **97**: E14-21.

**Hosoya T, Matsushima M, Nukariya K, Utsunomiya K.** The relationship between the severity of depressive symptoms and diabetes-related emotional distress in patients with type 2 diabetes. *Intern Med.* 2012; **51**: 263-9.

## Laboratory Animal Facilities

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Kiyoshi Ohkawa, *Professor and Director*

Adumi Wada, *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 2011, 167 researchers used the LAF. We undertake breeding of experimental animals and technically guide researchers in animal experimentation. In addition, we performed the following studies to develop basic medical sciences, including laboratory animal science.

### Research Activities

*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*

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. In medical science, the FOBT was developed as a screening test for alimentary canal tumors and inflammatory diseases. A chemical FOBT was based on the peroxidase activity of hemoglobin. Thus, this chemical test had low sensitivity and specificity, because it often obtained false-positive and a false-negative results if patients' diets contained hemoglobin of other species, myoglobin, and vitamin C. Therefore, a test subject must be placed on a restrictive diet before a chemical FOBT. For this reason, the FOBT is not widely used in veterinary practice. 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 novel 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 significant increase of FOBT values in dogs. This increase was significantly decreased with anthelmintic 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 collecting cases of gastrointestinal cancer in dogs. In addition, we are analyzing changes over time after experimental infection of *Echinococcus multilocularis* in dogs.

*Establishment and characterization of strains derived from Japanese wild mice and Phodopus hamster*

Our inbred strains derived from Japanese wild mice (*Mus musculus molossinus*) and *Phodopus* hamsters were maintained in this laboratory.

Japanese wild mice are classified as *M.m.molossinus* and originated from a natural inter-subspecific hybrid between *Mus musculus castaneus* inhabiting southwest Asia and *Mus musculus musculus* distributed in north Asia. The *M.m.molossinus* subspecies is an excellent source for improving laboratory mice, because it was suspected to be greatly different in gene constitution from common laboratory mice derived from the *Mus musculus domesticus* subspecies. We have established several new inbred strains based on *M.m.molossinus* mice captured in Osaka Prefecture. These strains are being maintained in our laboratory, and new consomic strains based on these strains are being developed. In collaboration with the Department of Molecular Biology, we developed 2 new mouse strains using our original *M.m.molossinus* inbred strain named MSKR. One is the congenic strain having knockout allele of *Oaz1* derived from the B6.129-*Oaz1*<sup>tm</sup> to the MSKR background, and the other is a consomic strain that has chromosome 10 derived from the above-mentioned strain to the MSKR background. We have confirmed that these newly established strains are useful for researching genetic modifications in *Oaz1* knockout mice.

The *Phodopus* hamster is a small rodent that differs taxonomically from the Syrian hamster, which is the major laboratory hamster. We recently determined that this hamster is a good candidate for a new laboratory animal and have established an inbred strain named PMI.

A PMI hamster with a morphologically abnormal stomach was found on anatomical screening in May 2010. Pathological observation suggested this abnormality represented a well-differentiated adenocarcinoma. By successive anatomical screening of 41 PMI hamsters, we confirmed the occurrence of morphologically abnormal stomachs in 39 of 41 hamsters (95.12%) at a mean age of 309 days (range, 196 to 515 days). On the other hand, all 12 hamsters of the inbred TAK strain, which was established from *Phodopus campbelli* in 2009, showed normal stomachs at a mean age of 256 days (range, 236 to 293 days). The high incidence of morphologically abnormal stomachs was thought to be a special feature of the PMI inbred strain.

#### *The search using the NC/Nga inbred strain for a novel drug for treating atopic dermatitis*

The NC/Nga inbred strain is the current mouse model for atopic dermatitis. However, the onset rates of dermatitis differ among separate lines in each laboratory. The NC/Nga inbred strain maintained in our laboratory has an extremely severe dermatitis diathesis. In collaboration with the Department of Tropical Medicine, we are using the NC/Nga mice to research new drugs for treating atopic dermatitis.

#### Publications

Wada A, Kanai T<sup>1</sup>, Ohkawa K, Tsudzuki M<sup>2</sup> (<sup>1</sup>Tokyo Women's Med Univ, <sup>2</sup>Hiroshima Univ). Stomachic carcinogenesis in the inbred hamster originated from *Phodopus campbelli*. *Exp Anim*. 2011; **60**: 262.

Kanai T<sup>1</sup>, Wada A, Ohkawa K, Tsudzuki M<sup>2</sup> (<sup>1</sup>Tokyo Women's Med Univ, <sup>2</sup>Hiroshima Univ). A case of Gastric carcinoma of the inbred Hamster from originated *Phodopus campbelli*. *Exp Anim*. 2011; **60**: 312.

## Radioisotope Research Facility

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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 nonradioisotopic research. 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 2011, 35 researchers from 14 departments and 14 students of 2 curriculums used the laboratory of this facility. Major nuclides used for experiments were  $^{32}\text{P}$ ,  $^{51}\text{Cr}$ ,  $^{125}\text{I}$ ,  $^{35}\text{S}$ , and  $^3\text{H}$ .

### Research Activities

#### *The active site of exfoliative toxin A of Staphylococcus aureus*

The exfoliative toxin (ET) produced by *S. aureus* causes staphylococcal scalded-skin syndrome. To determine the biologically active site of ETA, we introduced mutations into specific Tyr residues of the *eta* gene using site-directed *in-vitro* mutagenesis. The mutant plasmids were used to express cETA in *E. coli* C6008S. While the exfoliative activity in neonatal mice and the antigenic activity of native cETA were as high as those of sETA produced by *S. aureus*, the cETAs of the Tyr mutant showed decreased toxicity and immunoreactivity, except for Tyr-161. Replacement of Tyr-17-18 or Tyr-225-232Tyr-41-43 of cETA led to complete loss of toxicity and immunoreactivity. These results suggest that this cluster of Tyr residues is essential for the toxicity and immunoreactivity of ETA.

#### *Development of techniques for determining radioactivity*

Radon is the second most common cause, after smoking, of lung cancer in the general population. Liquid scintillation counting has been a standard method for measuring radon in air and water. We have reported the determination of radon in water and air samples using a methylphenyl silicone oil scintillator. Radon in water was extracted with the silicone oil scintillator and with a toluene scintillator. A silicone rubber scintillator has been made from the materials KER-6150-A and B (Shin-etsu Chemical Co., Ltd., Tokyo, Japan) to determine the radon concentration in air. The silicone rubber scintillator is transparent, flexible, water-resistant, heat-resistant, and cold-resistant.

#### *Analysis of resistance mechanisms in radiation-resistant organisms*

Tardigrades show remarkable adaptability in extreme environmental conditions, such as high radiation, high temperature, and high pressure. Tardigrades isolated from activated sludge were identified with 18S ribosomal DNA as being of the genus *Isohypsibius*. Tardigrades stained with a fluorescent probe (CellTracker Green CMFDA, Molecular Probes,

Life Technologies Corp., Carlsbad, CA) were irradiated with  $^{60}\text{Co}$  with doses of 500, 1,000, 2,000, 4,000, and 7,000 Gy at the Takasaki Advanced Radiation Research Institute (Takasaki, Gunma Prefecture). When tardigrades were not stained, survival rates with doses of 1,000 Gy or less were as high as those without radiation. However, tardigrades that were stained had much lower survival rates after being irradiated with low doses. These results suggest that fluorescent staining has a radiosensitizing effect on *Isohypsibius*.

#### *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. More than 70 soil samples were collected from April through November 2011 in the Kanto area. Leaves (pine, Japanese cedar, cypress, and bamboo) and bamboo shoots were also examined with radiation images using an imaging plate system. Cesium-134 and cesium-137 were detected in all samples, and iodine-131 was detected in some samples. At the time of sampling the concentrations of cesium-134 and cesium-137 in soil samples in the Kanto area ranged from 0.4 to 100 kBq/m<sup>2</sup> and from 0.5 to 130 kBq/m<sup>2</sup>, respectively.

#### **Publications**

**Shinji H, Yosizawa Y, Tajima A, Iwase T, Sugimoto S, Seki K, Mizunoe Y.** Role of fibronectin-binding proteins A and B in in vitro cellular infections and in vivo septic infections by *Staphylococcus aureus*. *Infect Immun.* 2011; **79**: 2215-23.

#### **Reviews and Books**

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## Core Research Facilities

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Yoshinobu Manome, *Professor and Director*  
Takeo Iwamoto, *Associate Professor*

Hiroyuki Sasaki, *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.

#### *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 registrant's experiment, if necessary.

#### *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

#### *Application of a monoclonal antibody to thyroid papillary carcinoma for bioimaging and high-sensitive assay of tumor cells*

A monoclonal antibody developed at our university specifically recognizes an antigen expressed in differentiated papillary thyroid carcinoma cells. Because the antibody is considered useful for detecting early lesions or metastases of thyroid carcinoma, a method

for determining trace amounts of antigen was developed. Although a method for labeling the antibody is being developed, the coupling efficiency remains unsatisfactory for immunoglobulin M molecules, such as our antibody, and, therefore, a simple and corroborative approach has been required. Previously, we coupled the antibody to quantum dots for immunofluorescent assay or bioimaging. This year, we devised a sandwich assay for highly sensitive detection or quantification of the antigen. Because even a small amount of the antigen can be quantified with our method, we started a clinical study using the antibody.

#### *Functional analysis of tight junctions in the epidermis*

Tight junctions (TJs) among adjacent epithelial cells control the paracellular permeability of solutes. Epidermal TJs restricts molecular movement to assist the stratum corneum as a secondary barrier in the skin. However, the role of TJs in molecular distribution in the epidermis has not been studied in detail. Calcium ions ( $\text{Ca}^{2+}$ ) induce keratinocyte differentiation and distribute to form a vertical gradient that peaks at the stratum granulosum. The stratum corneum forms the  $\text{Ca}^{2+}$  gradient because it is considered the only permeability barrier in the skin. However, the epidermal TJs in the stratum granulosum have recently been suggested to restrict molecular movement to assist the stratum corneum as a secondary barrier. The objective of this study was to clarify the contributions of TJs to the  $\text{Ca}^{2+}$  gradient and to epidermal differentiation in reconstructed human epidermis. When the epidermal TJ barrier function was disrupted by treatment with sodium caprate,  $\text{Ca}^{2+}$  flux increased and the gradient changed in ion-capture cytochemical images. Ultrastructural changes and alterations of proliferation/differentiation markers revealed that both hyperproliferation and precocious differentiation occurred regionally in the epidermis. These results suggest that TJs play a crucial role in maintaining epidermal homeostasis by controlling the  $\text{Ca}^{2+}$  gradient.

#### *Study of interaction between staphylococcal exfoliative toxin A and ganglioside GM1 using surface plasmon resonance*

Several molecular species of gangliosides have been shown to act as receptors for various bacterial toxins. To date, various analytical methods, such as inactivation of toxic activity, inhibition of toxin binding, and changes in mobility on polyacrylamide gel electrophoresis, have been reported. We now describe the formation of intermolecular complexes between ganglioside GM1 and staphylococcal exfoliative toxin A (sETA) using the surface plasmon resonance technique, native polyacrylamide gel mobility shift assay, and the sETA toxicity inactivation test in the epidermis of newborn mice. The electrophoretic pattern of sETA showed a single faster band than control sETA when 4.5  $\mu\text{g}$  of sETA was mixed with 62.5 to 500  $\mu\text{g}$  of GM1; also sETA toxicity was completely abolished when 4.5  $\mu\text{g}$  of sETA was incubated with 1.25 mg of GM1. Affinities between sETA and GM1 were determined with a Biacore 3000 surface plasmon resonance analysis system (GE Healthcare, Chalfont St. Giles, UK) with a CM5 sensor chip on which sETA was immobilized through amine-coupling chemistry. The association and dissociation rates and affinity constants of GM1 were calculated using the BIAevaluation software kit, version 3.1 (GE Healthcare). Kinetic analysis showed that the association and dissociation rate

constants of the binding interaction of GM1 to sETA were  $5.6 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$  and  $5.6 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ , respectively, and the dissociation constant was 4.6 nM at 25°C. The present results demonstrate the high specificity of the interaction between GM1 and sETA.

## Publications

**Takeyama H, Kyoda S, Okamoto T, Manome Y, Watanabe M, Kinoshita S, Uchida K, Sakamoto A, Morikawa T.** The expression of sialic fibronectin correlates with lymph node metastasis of thyroid malignant neoplasmas. *Anticancer Res.* 2011; **31**: 1395-8.

**Watanabe M, Fujioka K, Akiyama N, Takeyama H, Manabe N, Yamamoto K, Manome Y.** Conjugation of quantum dots and JT95 IgM monoclonal antibody for thyroid carcinoma without abolishing the specificity and activity of the antibody. *IEEE Trans Nanobioscience.* 2011; **10**: 30-5.

**Takahara A, Koido S, Ito M, Nagasaki E, Sagawa Y, Iwamoto T, Komita H, Ochi T, Fujiwara H, Yasukawa M, Mineno J, Shiku H, Nishida S, Sugiyama H, Tajiri H, Homma S.** Gemcitabine enhances Wilms' tumor gene WT1 expression and sensitizes human pancreatic cancer cells with WT1-specific T-cell-mediated antitumor immune response. *Cancer Immunol Immunother.* 2011; **60**: 1289-97.

**Inaba N, Kimura M, Fujioka K, Ikeda K, Somura H, Akiyoshi K, Inoue Y, Nomura M, Saito Y, Saito H, Manome Y.** The effect of PTEN on proliferation and drug- and radiosensitivity in malignant glioma cells. *Anticancer Res.* 2011; **31**: 1653-8.

**Inaba N, Fujioka K, Saito H, Kimura M, Ikeda K, Inoue Y, Ishizawa S, Manome Y.** Down-regulation of EGFR prolonged cell growth of glioma but did not increase the sensitivity to temozolomide. *Anticancer Res.* 2011; **31**: 3253-7.

**Takeyama H, Shimada T, Manome Y, Uchida K, Morikawa T.** Detection of micrometastatic

cells in peripheral blood and bone marrow fluid of stage I-III Japanese Breast Cancer Patients and Transition following anti-cancer drug treatment. *Breast J.* 2012; **18**: 85-7.

**Sato K, Yokosuka S, Takigami Y, Hirakuri K, Fujioka K, Manome Y, Sukegawa H, Iwai H, Fukata N.** Size-tunable silicon/iron oxide hybrid nanoparticles with fluorescence, superparamagnetism and biocompatibility. *J Am Chem Soc.* 2011; **133**: 18626-33.

**Funamizu N, Kamata Y, Misawa T, Uwagawa T, Lacy CR, Yanaga K, Manome Y.** Hydroxyurea decreases gemcitabine resistance in pancreatic carcinoma cells with highly expressed ribonucleotide reductase. *Pancreas.* 2012; **41**: 107-13.

**Fujioka K, Shirasu M, Manome Y, Ito N, Kakishima S, Minami T, Tominaga T, Shimozono F, Iwamoto T, Ikeda K, Yamamoto K, Murata J, Tomizawa Y.** Objective display and discrimination of floral odors from *Amorphophallus titanum*, bloomed on different dates and at different locations, using an electronic nose. *Sensors.* 2012; **12**: 2152-61.

**Sakurai S, Iwamoto T.** Study of the interaction between sETA and ganglioside GM1 using surface plasmon resonance. *Archives and Bulletin of Kohno Clinical Medicine Research Institute.* 2011; **27**: 1-9.

**Fujioka K, Hanada S, Kanaya F, Hoshino A, Sato K, Yokosuka S, Takigami Y, Hirakuri K, Shiohara A, Tilley RD, Manabe N, Yamamoto K, Manome Y.** Toxicity test: Fluorescent silicon nanoparticles. *J Phys Conf Ser.* 2011; **304**: 012042.

## Department of Genetic Diseases and Genome Science

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Yoshikatsu Eto, *Professor and Director*  
Takashi Higuchi, *Postdoctoral Fellow*  
Torayuki Okuyama, *Visiting Professor*

Collaborating Researchers  
Hiroyuki Ida, *Professor*  
Toya Ohashi, *Professor*  
Hiroshi Kobayashi, *Assistant Professor*  
Masayuki Kobayashi, *Assistant Professor*

### General Summary

The main research topics in the Department of Genetic Diseases and Genome Science are the basic pathogenesis of genetic diseases, particularly, lysosomal storage diseases (LSDs), and the development of therapies for LSDs. Of our research topics, the pathogenesis of central nervous system (CNS) involvement in LSDs is the most important. To understand the pathophysiology of CNS events in LSDs, we generated induced pluripotent stem (iPS) cells from mucopolysaccharidosis (MPS) VII mice and caused them to differentiate into neuronal cells. We also generated iPS cells from a mouse model of Pompe disease and caused them to differentiate into skeletal muscle cells. We can produce disease models of various LSDs using iPS technology. Furthermore, we treated CNS involvement of LSDs by means of intrathecal injection of enzymes into MPS II mice. These findings indicate that intrathecal treatment is feasible for treating the CNS in various LSDs.

### Research Activities

1. iPS cells from various LSDs are important research tools for understanding the pathophysiology of LSDs and can also be applied to the treatment of LSDs. We successfully generated iPS cells from Pompe mice by means of tail-tip fibroblasts, mouse embryonic fibroblasts, and 3 factors: Klf4, Sox2, and Oc2/4t. The iPS cells differentiated into skeletal muscle cells. Pompe skeletal muscle cells showed massive accumulation of glycogen in lysosomes surrounded by a single membrane unit. We also generated iPS cells from patients with Gaucher disease, Fabry disease, and Pompe disease.

2. Development of treatment procedures for LSDs

To establish novel treatment procedures for CNS involvement of LSDs is our most important project. One procedure is intrathecal or intraparenchymal injection of enzymes into MPS II mice. We found that intrathecal injection produced significant elevations of enzyme activities in various regions of the brain and in other organs, such as the liver, spleen, kidney, and heart. Furthermore, histological improvement in the brains of MPS II mice was also observed.

3. The screening for LSDs with dried blood spots is an important technology for the early diagnosis and treatment of patients with various LSDs. We used the fluorometric assay method to establish the dried blood spot method for the early diagnosis of Pompe disease, Fabry disease, Morquio syndrome, and MPS VI. Furthermore, we recently established a dried blood spot diagnostic method for Wolman disease.

## Publications

**Kawagoe S, Higuchi T, Meng XL, Shimada Y, Shimizu H, Hirayama R, Fukuda T, Chang H, Nakahata T, Fukada S, Ida H, Kobayashi H, Ohashi T, Eto Y.** Generation of induced pluripotent stem (iPS) cells derived from a murine model of Pompe disease and differentiation of Pompe-iPS cells into skeletal muscle cells. *Mol Genet Metab*. 2011; **104**: 123–8.

**Yokoi T, Kobayashi H, Shimada Y, Eto Y, Ishige N, Kitagawa T, Otsu M, Nakauchi H, Ida H, Ohashi T.** Minimum requirement of donor

cells to reduce the glycolipid storage following bone marrow transplantation in a murine model of Fabry disease. *J Gene Med*. 2011; **13**: 262–8.

**Shimada Y, Nishida H, Nishiyama Y, Kobayashi H, Higuchi T, Eto Y, Ida H, Ohashi T.** Proteasome inhibitors improve the function of mutant lysosomal  $\alpha$ -glucosidase in fibroblasts from Pompe disease patient carrying c.546G>T mutation. *Biochem Biophys Res Commun*. 2011; **415**: 274–8.

## Department of Allergology

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Naohiro Watanabe, *Professor and Director*

Hirohisa Saito, *Professor*

### General Summary

The Department of Allergology was established this year by donation. Our research concerned the biological significance of allergic immune reactions by immunoglobulin (Ig) E, mast cells, eosinophils, and basophils.

### Research Activities

#### *Mechanisms of atopic dermatitis*

We reported on the NC/Nga mouse as a model of spontaneous development of atopic dermatitis with hyper-IgE. Genetic analysis identified that dermatitis was controlled by 1 recessive gene and that hyper-IgE was controlled by 2 recessive genes. No linkage was found among these genes. Infection of mites was frequently found in NC/Nga mice raised under conventional conditions. Mite infection induced infiltration of mast cells and eosinophils in the skin. Numbers of mast cells and eosinophils in the skin of NC/Nga mice with dermatitis were significantly higher than those in mice without dermatitis. An inhibitor of mast cell-specific chymase developed by us suppressed the development of dermatitis and infiltration of mast cells and eosinophils in the skin of NC/Nga mice. One mechanism of these phenomena is the activation of stem cell factor by chymase. Activated stem cell factor is a major molecule for the differentiation of mast cells and for the chemotaxis of eosinophils. In addition, injection of chymase to the skin elicited eosinophil infiltration in mice. These results suggest that mast cells and chymase play roles in the development of atopic dermatitis.

#### *Dengue virus infection and mast cells*

Dengue virus infection is associated with several diseases, including Dengue fever (DF), Dengue hemorrhagic fever (DHF), and Dengue shock syndrome (DSS), by the criteria of the World Health Organization. Both DHF and DSS are severe forms of Dengue infection and are characterized by increased vascular permeability and hemorrhagic manifestations. To clarify the involvement of mast cells in severe Dengue diseases, levels of mast cell-derived mediators were measured in patients and control subjects in Vietnam. Levels of mast cell-specific chymase and tryptase were significantly higher in patients with DHF or DSS than in patients with DF or in control subjects. Serum levels of vascular endothelial cell growth factor derived from mast cells were significantly elevated in patients with DHF or DSS. When the patients had recovered, the levels of these mediators had returned to levels similar to those of control subjects. Levels of the mast-cell activators interleukin 9 and 17 were also increased in patients with DHF or DSS. These findings suggest that mast cells participate in the pathogenesis of DHF and DSS.

## Publications

**Ogata A<sup>1</sup>, Fujieda Y<sup>1</sup>, Terakawa M<sup>1</sup>, Muto T<sup>1</sup>, Maruoka H<sup>1</sup>, Nagahira K<sup>1</sup>, Fukuda Y<sup>1</sup>, Tomimori Y<sup>1</sup>, Watanabe N<sup>1</sup> (Asubio Pharma).** Pharmacokinetic/pharmacodynamic analyses of chymase inhibitor SUN138334 in NC/Nga mice and prediction of effective dosage for atopic dermatitis patients. *Int Immunopharmacol.* 2011; **11**: 1628-

32.

## Reviews and Books

**Watanabe N.** Search after new concepts on IgE (in Japanese). *Tokyo Jikeikai Ika Daigaku Zasshi.* 2011; **126**: 149-62.

## Department of Rehabilitation Medicine

### Division of Physical Fitness

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Masahiro Abo, *Professor and Director*

Hideki Yamauchi, *Assistant Professor*

#### General Summary

Research activities in our division have been focused on the plasticity of skeletal muscle in terms of exercise physiology.

#### Research Activities

##### *Effects of exercise training and protein malnutrition on skeletal muscle in growing rats*

We studied the effects of regular exercise and protein malnutrition in growing rats. Regular exercise caused myofiber hypertrophy, with a shift of the myosin heavy chain (MHC) isoform composition from type IIb-dominant to type IIa-dominant, and various metabolic adaptations: increases in the activities of citrate synthase and  $\beta$ -hydroxyacyl-coenzyme A dehydrogenase, and in expression levels of glucose transporter type 4 and fatty acid translocase/CD36. Levels of cholesterol in the serum of exercised rats were decreased. On the other hand, in sedentary rats daily intake of low-protein chow (5% protein) inhibited the growth of muscle mass without a shift in MHC isoform composition or metabolic adaptations. Serum cholesterol levels were increased in these rats. Even under the condition of protein malnutrition, however, regular exercise was found to induce myofiber hypertrophy and metabolic adaptations. These findings indicate that exercise is an intense stimulus for enhancing physical and health-related fitness. As for cellular signaling pathways, regular exercise induced a decrease in myostatin and increases in the expression of inhibitor of nuclear factor  $\kappa$ B  $\alpha$ , peroxisome proliferator-activated receptor- $\gamma$  coactivator (PGC) 1 $\alpha$ , and heat shock protein 60, and in the levels of phosphorylated adenosine monophosphate kinase (AMPK)  $\alpha$ , AMPK $\beta$ 1, and acetyl-coenzyme A carboxylase. These findings suggest that activation of AMPK is involved in the adaptations of carbohydrate and lipid metabolism and that the down-regulation of myostatin and of nuclear factor  $\kappa$ B signaling is involved in the myofiber hypertrophy induced by regular exercise.

##### *Effect of intermittent reloading on muscle atrophy induced by hindlimb unloading*

We examined cellular disorders in skeletal muscles atrophied with hindlimb unloading and the interventional effects of intermittent reloading in adult (5 months) and old (24 months) rats. Intermittent reloading was performed daily as 3 sessions of 10 minutes of exercise, every 4 hours (total, 30 minutes/day) during 3-week hindlimb-unloading period. Hindlimb unloading without intermittent reloading induced significant muscle atrophy and fiber degeneration in the lateral gastrocnemius. Both Akt activity (phospho-Akt/pan Akt ratio) and myostatin expression were decreased in the red regions but not in the white region of the muscle. These changes in the red regions were inhibited by

intermittent reloading in the old rats but not in the adult rats. Because the atrophy and fiber degeneration were more severe in old rats than in adult rats, unloading seems to have a larger effect in old rats than in adult rats. Furthermore, we believe that the degenerating effect of hindlimb unloading involves PGC-1, because its expression in the lateral gastrocnemius was in the order of hindlimb unloading group < hindlimb unloading plus intermittent reloading group < control group. As for the effect of intermittent reloading, expression of heat shock proteins may be involved. This is because the expression of heat shock proteins was in the order of hindlimb unloading group < control group < hindlimb unloading plus intermittent reloading group. In any event, the present results suggest that hindlimb unloading and intermittent reloading each stimulates individual cellular adaptation processes that are largely independent of each other.

## Department of Cell Physiology Division of Aerospace Medicine

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Satoshi Kurihara, *Professor*  
Hiroko Toshima, *Associate Professor*

Masamichi Sudoh, *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 electrocardiography in 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 aboard the International Space Station are now being prepared. Using the transparent medaka strain Sukesuke (SK2), we established a way to detect the heartbeat and to observe heart-rate variability with live imaging under a stereomicroscope. However, because there is no evidence that the live imaging data is coincident with electrocardiography (ECG), 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, Japan).

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 of 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.

*Results of physioepidemiological study*

Many previous studies of wellness medicine and occupational health have been performed with epidemiological methods. However, in this study physiological data (e.g., ECG) were analyzed with epidemiological methods. Mental stress and human health can be evaluated objectively using both physiological and epidemiological methods.

1. We studied the risk of cardiovascular disease due to smoking. We measured spontaneous platelet aggregation and whole-blood fluidity before and after smoking. Thrombophilia increased after smoking.
2. Mental stress in nurses during nursing practice. We directly measured the mental stress of nurses using a Holter ECG monitor while they practiced nursing. Their stress increased when patients died and when nurses supported bone marrow puncture. Moreover, when nurses were unskilled or were busy, their stress increased.

## Department of Orthopaedic Surgery

### Division of Sports Medicine

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Keishi Marumo, *Professor*

Hiroki Funasaki, *Associate Professor*

#### General Summary

##### *Clinical Research*

The ongoing research in our department concentrates 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 focused on basic research in 2011.

#### Research Activities

##### *The effect of the ankle-joint angle on training of peroneus muscles*

The purpose of this study was to evaluate the effect of the ankle-joint angle on training of the peroneus muscles. During eversion of the ankle joint, the observed activity of the peroneus muscles on electromyography (EMG) was more predominant in plantar flexion than in the neutral position. In healthy adults, larger ankle-eversion strength was obtained after tube-muscle training with the ankle in plantar flexion than in the neutral position. In patients with ankle-inversion sprain, peroneus strength was significantly lower on the affected side than on the unaffected side. After 2 months of tube-muscle training, muscle strength became nearly equal on both sides. These findings suggest that training of the peroneus muscles is more effective when performed with the ankle in plantar flexion.

##### *Three-dimensional gait analysis in patients with bilateral knee osteoarthritis before and after unilateral total knee arthroplasty*

The purpose of this study was to compare the data of 3-dimensional gait analysis with a motion analysis system (Vicon Motion Systems, Oxford, UK) in 26 patients with bilateral knee osteoarthritis who had undergone unilateral total knee arthroplasty. Analyzed variables were: 1) step length, 2) walking speed, 3) percentage of single limb support phase, 4) ground force during the single limb support phase, 5) step width, and 6) range of motion of the hip, knee, and ankle joint. Step length, walking speed, percentage of single limb support phase, ground force during the single limb support phase, and range of motion were significantly improved in patients with a Japanese Orthopaedic Association score of 60 points or more for the side not operated on. On the other hand, these variables did not improve in patients with a score of less than 60 points. We conclude that the various gait variables improve after total knee arthroplasty, although patients with severe osteoarthritis (on the nonoperated side) show no improvement in walking ability.

*The changes in the silent period before and after standing on a balance mat*

The purpose of this study was to evaluate the changes in premotion time (PMT), pre-motion silent period (PMSP), and switching silent period (SSP) before and after training with a balance mat. Twenty healthy persons were randomly divided into 2 groups: a control group and a balance-mat group. Activities of the soleus and tibialis anterior muscles were recorded with EMG while the subjects tried to raise both heels quickly in response to a flashing light. Intervention consisted of 3 minutes of standing on the floor in the control group and of 3 minutes of standing on the balance-mat in the balance-mat group. Then, EMG was recorded in the same manner after the intervention in each group. We found no significant difference in the PMSP or SSP between the groups before intervention. On the other hand, after intervention the PMSP and SSP were significantly shorter in the balance-mat group than in the control group. In addition, in the balance-mat group, the PMSP and SSP were significantly shorter after intervention than before intervention. There was no statistical difference in the PMT between before and after intervention. These results suggest that balance-mat training is effective for shortening the SSP and PMSP, which lead to control of the posture function.

*Femoral condyle irregularity in athletes during growth period*

We reported on 3 young soccer players with femoral condyle irregularity of the knee. These cases were similar to osteochondritis dissecans regarding magnetic resonance findings, location, and age at onset. Careful attention should be paid to femoral condyle irregularity; if magnetic resonance is performed for young patients, an unnecessary interruption of sports activity can be avoided.

*Treatment for the elderly golfers in our clinic*

We evaluated 170 patients who were older than 40 years and had visited our clinic in the last 3 years. The majority of the patients had adhesive capsulitis of the shoulder or osteoarthritis of the lumbar spine or the knees. With conservative therapy, the majority of them resumed sports activities within 2 months. In addition, athletic rehabilitation, such as exercise to increase range of motion and balance training, were also performed and was useful for these patients. Rehabilitation should be continued even after patients resume their sports activities.

## Publications

**Funasaki H, Yoshida M, Kan I, Kato S, Kasama K, Marumo K.** Arthroscopic surgery for traumatic anterior instability of the shoulder with general joint laxity (in Japanese). *Katakansetsu*. 2011; **35**: 357-60.  
**Yoshida M, Funasaki H, Kato S, Kasama K, Marumo K.** Gene expression analysis for the synovium of glenohumeral joint in primary frozen shoulders (in Japanese). *Katakansetsu*. 2011; **35**: 613-6.  
**Iwama T, Yoneda S, Marumo K, Funasaki H, Roppongi S, Kajiwara M, Ishii M, Kinoshita K.**

Guidance on the throwing form using illustrations (in Japanese). *Nihon Rinsho Supotsu Igakkaishi*. 2011; **19**: 460-5.  
**Hayashi H, Funasaki H, Roppongi S, Oda H, Kato H, Marumo K.** Dislocation fracture of the Lisfranc joint in a young soccer player (in Japanese). *Nihon Seikeigeka Supotsu Igakkai Zasshi*. 2012; **32**: 34-7.

## Reviews and Books

**Funasaki H.** Management for low back pain and

lumbar disease (in Japanese). *Sogo Rihabirite-shon*. 2011; **39**: 853-7.

**Funasaki H.** Orthopaedic problems in neurofi-

bromatosis type I patients (in Japanese). *Nihon Rekkuringuhauzenbyo Gakkai Zasshi*. 2011; **2**: 15-9.

## Health-Care Center

---

Mikio Zeniya, *Professor and Director*  
 Takashi Wada, *Professor*  
 Hiroki Takahashi, *Assistant Professor*  
 Yuji Kuniyasu, *Assistant Professor*

Yoichi Sakamoto, *Professor*  
 Takekazu Onda, *Professor*  
 Tsutomu Fukumoto, *Assistant Professor*  
 Hiroko Nogi, *Assistant Professor*

### General Summary

#### *Shimbashi Medical Checkup Office*

The serum level of alkaline phosphatase (ALP) is a basic test variable adopted by the Japan Society of Ningen Dock. ALP varies depending on age, sex, blood group, and menstrual status, but the extent of its variation depending on these factors has not been fully clarified. Our study was aimed at evaluating the effects of age, sex, blood group, and menstrual status on ALP.

### Research Activities

#### *Shimbashi Medical Checkup Office*

ALP is used as an indicator of hepatic and biliary tract disorders. The present study was designed to evaluate the effects of age, sex, blood group, and menstrual status on ALP.

1. Age and sex: A total of 4,263 subjects (2,835 men and 1,428 women) aged 40 to 59 years were studied. These subjects were free of hepatopathy, hepatitis B or hepatitis C virus infection, and nephropathy. Subjects who affirmed on a questionnaire that they had bone disease or thyroid disease or were pregnant were excluded from the study. The subjects were divided into 4 age groups to analyze intergroup differences: group 1 (40–44 years), group 2 (45–49 years), group 3 (50–54 years), and group 4 (55–59 years). Among men, the ALP levels were 195, 198, 198, and 196 U/L in groups 1, 2, 3, and 4, respectively, and showed no age-related changes. Among women, the ALP levels increased with age and were 153, 164, 195, and 226 U/L in groups 1, 2, 3, and 4, respectively. The ALP level at age 40 to 54 years was lower for women than for men, whereas the ALP level at age 55 to 59 years was higher for women than for men.

2. Blood group and menstrual status: Of the women mentioned above, 389 aged 45 to 54 years receiving gynecologic examinations were studied. Women aged 45 to 54 years were divided on the basis of blood group. Each group was subdivided on the basis of menstrual status: premenopause group (mean age, 50 years), less than 3-year menopause group (less than 3 years after menopause), and more than 3-year menopause group (more than 3 years after menopause). The ALP levels in the premenopause group, the less than 3-year menopause group, and the more than 3-year menopause group were 151, 209, and 261 U/L among women with blood type A; 165, 204, and 207 U/L among women with blood type B; 142, 245, and 232 U/L among women with blood type AB female; and 180, 201, and 234 U/L among women with blood group O, respectively. Thus, the ALP level was higher in the less than 3-year menopause group than in the premenopause group among women with each blood type other than type O, and the ALP level was higher in

the more than 3-year menopause group than in the premenopause group among women with each blood type. Next, differences in ALP levels according to blood type were analyzed in each menstrual status group. In the premenopause group, the ALP levels were 151, 165, 142, and 180 U/L for women with blood types A, B, AB, and O, respectively, indicating a higher ALP level in women with blood type O than in women with blood type A or AB. In the less than 3-year menopause group, the ALP levels were 209, 204, 245, and 201 U/L for women with blood type A, B, AB, or O. In the more than 3-year menopause group, the ALP levels were 261, 207, 232, and 234 U/L, respectively. Thus, the ALP level before menopause was higher in women with blood type O than in women with blood type A or AB, but such differences in the ALP level among blood types were absent after menopause. The results can be summarized as follows.

- 1) The ALP level in men did not change with age. The ALP level in women was lower than that in men at age 40 to 54 years but increased sharply after age 50 years and was higher than that in men at age 55 to 59 years.
- 2) The ALP level in women less than 3 years after menopause (excluding women with blood type O) and women more than 3 years after menopause was lower than that in women before menopause, suggesting an association of menopause with the ALP level.
- 3) Before menopause, the ALP level was higher in women with blood type O than in women with blood type A or AB. After menopause there was no difference in the ALP level depending on blood type.

## Publications

**Takahashi H, Zeniya M.** Acute presentation of autoimmune hepatitis: Does it exist? A published work review. *Hepatol Res.* 2011; **41**: 498-504.

**Abe M, Mashiba T, Zeniya M, Yamamoto K, Onji M, Tsubouchi H, Autoimmune Hepatitis Study Group-Subgroup of the Intractable Hepato-Biliary Disease Study Group in Japan.** Present status of autoimmune hepatitis in Japan: a nationwide survey. *J Gastroenterol.* 2011; **46**: 1136-41.

**Hayashi K, Ichirizuka T, Fukumoto T, Joki M,**

**Inaji J, Toyohara K, Nakazaki K, Mashima K, Zeniya M, Wada T.** Investigation of life-style factors that worsen lung age in non-smoker (in Japanese). *Ningen Dock.* 2011; **26**: 87-93.

**Wada T, Yamakado M, Ishizaka Y, Munakata S, Murohara T, Nakamura M, Fukuda K, Igarashi A.** No smoking guide and the investigation of the no smoking action in the medical checkup facilities of ningen dock (in Japanese). *Ningen Dock.* 2011; **26**: 627-37.

## Premedical Course

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### Biology

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Osamu Terasaka, *Professor*

Rie Hiratsuka, *Assistant Professor*

#### General Summary

The main research subject of our laboratory is the reproductive system of seed plants. Our research is now focused on the relation between pollen tube growth and the programmed cell death of pollen tube conducting tissue.

#### Research Activities

*Nonformation of plasmodesmata is involved in prothallial cell death during Gymnosperm pollen development*

In the development of gymnosperm pollen, a small prothallial cell is cut off from the main body of the spore through unequal cell division. In *Ginkgo biloba*, two prothallial cells were produced; the first (p1) died, and the second (p2) survived. Immediately after formation, a nucleus with regularly dispersed chromatin and some organelles was included in both p1 and p2. Subsequently, p1 degraded rapidly and exhibited a uniformly high electron density. Vacuole development and collapse, a main trigger of cell death in plants, was not observed. The cell walls of p1 and its sister cell, the embryonal cell, became thickened, and no plasmodesmata formed between them. However, the walls of p2 and its sister cell, the antheridial initial, were thin with some plasmodesmata between them. In *Pinus thunbergii*, 2 prothallial cells also formed in the pollen; however, both cells died in a manner similar to that of p1 of *G. biloba*. They were TUNEL-positive and did not form plasmodesmata. In pollen of *P. thunbergii*, surviving prothallial cells were induced artificially by centrifugal treatment that perturbed the polarity or inequality or both of division in normal pollen mitosis. Some of these cells formed plasmodesmata with adjoining sister cells. These results suggest that cell death in prothallial cells is not “vacuolar cell death” and that nonformation of plasmodesmata, which facilitates the transport of materials between cells, affects prothallial cell death.

#### Publications

**Terasaka O, Hiratsuka R.** A new pattern of phragmoplast growth brings about asymmetric cell

division in the pollen of Ephedra. *Nihon Kafun Gakkai Kaishi*. 2011; **57**: 5–15.

## Physics

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Tsuyoshi Ueta, *Professor*

Katsumi Kasono, *Assistant Professor*

### General Summary

1. Since 1998, by introducing lattice vibration in photonic crystals artificially, we have investigated the direct interaction between an incident light and lattice vibration and have found that the incident light is amplified. We have proposed a metal photonic crystal as a system for enhancing the dynamic Casimir effect, and have been investigating the properties of the dynamic Casimir effect within a metal photonic crystal.
2. Phase transitions, critical phenomena, interacting many-body systems, computer simulation.

### Research Activities

#### *The photon-phonon interaction within a lattice-vibrating photonic crystal*

In 2011, the phenomenon of photon-phonon interaction has been investigated within metal photonic crystals, and the very strong resonance amplification of an incident light has been found. We have presented our results at the International Conference on Materials for Advanced Technologies 2011 and have also published a paper in a journal.

#### *Monte Carlo simulations of the ferromagnetic Potts models*

We calculated the discontinuity of magnetization 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, dice, and triangular lattices. The results show that discontinuity of magnetization has no universality between the different lattices.

### Publications

**Fujii G<sup>1</sup>, Matsumoto T<sup>1</sup>, Takahashi T<sup>1</sup>, Ueta T<sup>1</sup> (Nagoya Univ).** Finite-element analysis of lasing modes within photonic random media. *J Phys B At Mol Opt Phys*. 2012; **45**: 085404. Epub 2012 Mar 30.

**Fujii G<sup>1</sup>, Matsumoto T<sup>1</sup>, Takahashi T<sup>1</sup>, Ueta T<sup>1</sup> (Nagoya Univ).** Study on transition from photonic-crystal laser to random laser. *Opt Express*. 2012; **20**: 7300-15.

**Fujii G, Matsumoto T, Takahashi T, Ueta T.** A study on effect of filling factor for laser action in dielectric random media. *Appl Phys A Mater Sci Process*. 2012; **107**: 35-42. Epub 2012 Jan 6.

**Ueta, T.** Two-dimensional electron systems in magnetic fields: the current equipartition law. *Advances in Condensed Matter Physics*. 2011; **2011**: 104843.

**Ueta T.** Amplification of light in one-dimensional vibrating metal photonic crystal. *Appl Phys A*

*Mater Sci Process*. 2012; **107**: 55-9. Epub 2011 Dec 24.

**Fujii T, Matsumoto T, Takahashi T, Yamada T, Ueta T.** Study on electric intensity dependency of laser action in randomly distributed dielectric rod (in Japanese). *Denkigakkai Ronbunshi C*. 2012; **132**: 89-95.

**Ueta T.** FDM Analysis of quantum transport properties in microwave with mathematica (in Japanese). *Keisan Suri Kogaku Ronbunshu*. 2011; **11**: 7-12.

**Fujii G, Matsuda H, Matsumoto T, Takahashi T, Yamada T, Ueta T.** Finite element analysis for laser action in honeycomb photonic crystals with random dangling bonds (in Japanese). *Keisan Suri Kogaku Ronbunshu*. 2011; **11**: 89-94.

**Ueta T.** Lattice vibration frequency dependence of amplification of light in a 1D metal photonic crystal (in Japanese). *Nihon Kikai Gakkai Keisan*

*Rikigaku Koenkai Ronbunshu*. 2011; **24**: 1309.  
**Fujii G, Matsumoto T, Takahashi T, Yamada T, Ueta T.** A study on the transition from photonic-

crystal laser to random laser (in Japanese). *Nihon Kikai Gakkai Keisan Rikigaku Koenkai Ronbunshu*. 2011; **24**: 1303.

## Chemistry

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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

#### *Theoretical analysis of molecular interactions of Cu(II) bis(diarylpropanedione)dicarboxylate complexes with benzene ligands*

Aene-polyfluoroarene interaction was found between the Cu(II) bis[3-oxo-1,3-bis(pentafluorophenyl)prop-1-en-1-olate] complex and benzene as the guest molecule. Density function theory (DFT) calculation suggested a weak interaction was present between the fluorine-containing aryl diketonate complexes of a specific conformation and the guest molecule. However, DFT calculation is not able to evaluate molecular interactions precisely. Second-order Møller-Plesset (MP2) calculations are more reliable, although the computation time for such a large molecule is long. To reduce MP2 computation time, more concise model complexes, acetylacetonate and hexafluoroacetylacetonate, were used. The MP2 calculation predicted Cu<sup>2+</sup>- $\pi$  interaction in the fluorinated complex more precisely, although MP2 calculations of large complexes with fluorinated aryl ligands would take several years.

#### *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 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 salts are the most effective among the carboxylate salts of amino acids tested for coupling with butoxycarbonate-amino acid active esters in an organic solvent, such as dimethylformamide or dimethylsulfoxide.

## Publications

**Hori A<sup>1</sup>, Kikuchi T<sup>1</sup>, Miyamoto K<sup>1</sup>, Okano T, Kachi-Terajima C<sup>2</sup>, Sakaguchi H<sup>1</sup> (Kitasato Univ, <sup>2</sup>Toho Univ).** Transformation of a cull thiazolo-1,2,4-triazine derivative from a metastable

coordination network to a monomer in solution and vapor conditions. *Eur J Inorg Chem.* 2011; **2011**: 3059–66.

## Social Science (Law)

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Ryuichi Ozawa, *Professor*

### General Summary

Problems of constitutional law in present-day Japan

### Research Activities

I presented “Reexamination of Constitutional Principles of Finance” at a symposium of the Japan Association of Public Finance Law (May 28, 2011).

## Publications

**Ozawa R.** Democratic Party's government and parliamentary democracy (in Japanese). *Nihon no Kagakusha.* 2011; **46**(7): 47–53.

**Ozawa R.** Democratic Party's government and future parliamentary democracy (in Japanese). *Gyozaisei Kenkyu.* 2011; **80**: 22–31.

**Ozawa R.** Posting at private apartment house and law (in Japanese). *Horitsujiho.* 2012; **84**(2): 69–73.

### Reviews and Books

**Ozawa R.** Future of Diet and local parliament (in

Japanese). In: Kashiwabara H. *Jichitai populism wo tou.* Tokyo: Jichitai kenkyusha; 2012. p. 73–102.

**Ozawa R.** Why Diet can't deal with? (in Japanese) In: Mori H, Shirafuji H, Aikyou K, editors. *3.11 to kenpo.* Tokyo: Nihon Hyoronsha; 2012. p. 69–77.

**Ozawa R.** Reexamination of constitutional principles of finances (in Japanese). In: Nihon Zaisei-hogakkai, editor. *Zaisei kenpo no saikento.* Tokyo: Zenkoku Kaikeshokuin Kyokai; 2012. p. 32–46.

## Human Science

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Takao Fukuyama, *Professor*

### General Summary

The study of Western philosophy and ethics

## Research Activities

### *Essential Encounter*

An encounter provides an impact, 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? 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 keep a proud-hearted attitude.

## Japanese

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Ikuko Noro, *Professor*

## General Summary

### *Development of suitable documents for patients written in Japanese*

I conducted studies to develop suitable documents for patients written in Japanese as a member of the "Project for Patient-Friendly Documents," which is a part of "Empirical Research and Action Research for the Development of the Patient-Professional Relationship in a New Paradigm Aiming at 'Thinking Health Together'," supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology.

### *A study of the effects on patients of the comprehensibility of oral explanation and physician attitudes during informed consent*

A survey was performed to investigate how physicians' attitudes during verbal explanations of informed consent, as well as the comprehensibility of those explanations, affect understanding, emotion, and decision-making in patients of varying ages.

## Research Activities

### *Development of suitable documents for patients written in Japanese*

First, our project group developed "A Manual for Physicians to Write Suitable Documents

for Patients,” in which important points were explained for writing comprehensible documents for patients. Second, we asked 30 physicians to write documents by referring to the manual. Third, we performed a survey to investigate how patients and physicians assessed these documents. I presented the results at the “Thinking Health Together” Symposium held at Rikkyo University in November 2011.

*A study of the effects on patients of the comprehensibility of oral explanation and physician attitudes during informed consent*

Our research revealed that patients’ understanding was affected primarily by the comprehensibility of verbal explanations. However, the level of understanding was also affected by physician attitudes, which were also the primary factor affecting patients’ emotions during the encounter. Effects on decision-making varied with age: young patients’ decision-making was affected by comprehensibility, whereas decision-making by middle-aged and older patients was affected more by physician attitude. Both comprehensible explanations and a friendly attitude are crucial for appropriate informed consent. I presented these results at the Medical Communication Symposium held at University of Tokyo in October 2011.

### Reviews and Books

**Noro I, Abe K, Ishikawa H.** The Roter Method of Interaction Process Analysis System (RIAS) (in

Japanese). 2nd ed. Nagoya: Sankeisha; 2011.

## Mathematics

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Katsuya Yokoi, *Professor*

Hiroshi Shiraishi, *Assistant Professor*

### General Summary

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

### Research Activities

- I. We summarized the properties of nonperiodic points in an infinite omega-limit set of interval maps. We studied omega-limit sets of nonautonomous discrete dynamic systems.
- II. We discussed a resampling procedure in the estimation of optimal portfolios when the financial returns are a class of time-varying autoregressive conditional heteroskedasticity processes. On the basis of the bootstrap method, we constructed a mean-variance optimal portfolio estimator and derived its asymptotic property.

## Reviews and Books

**Yokoi K.** Non-periodic points in an infinite omega-limit set: a theorem of Sarkovskii (in Japa-

nese). *Kyoto University, Kyoto Daigaku Suri Kai-seki Kenkyusho Kokyuroku*. 2012; **1781**: 108-10.

## English

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Osamu Ohara, *Professor*

Tetsuro Fujii, *Associate Professor*

### General Summary

*English audiovisual education and the history of the English language (Ohara)*

*English Language communication and education: Material analysis and development (Fujii)*

Ohara continued his study of graphology and morphology in the letters of the Celys and the Stonors of the fifteenth century. Ohara also continued a study of how to make useful digital images and XML files of fifteenth century manuscripts, especially of the *Stonor Letters*.

Fujii joined a project team to compile English textbooks for high school English classes: English Communication I, II, and III. In addition to compiling the textbooks, Fujii is working on teacher's manuals and exercise materials. He studied example sentences in English learners' dictionaries and identified the types of sentences that are conducive to learning.

### Research Activities

Ohara visited the National Archives in the United Kingdom and studied the *Stonor Letters*. Making use of the results of the study, Ohara continued his research of the graphemes of the letters of the Stonors.

Fujii analyzed and collected authentic English materials to meet the level and the needs of high-school textbooks based on current teaching methodologies, theories, and research findings on learning English as a foreign language. These materials were used to compile textbooks following the revised teaching guidelines set out by the Ministry of Education, Culture, Sports, Science and Technology. The first textbook in the series, *World Trek—English Communication I*, received approval from the Ministry and is to be used in high schools from April 2013.

Fujii presented about learner-friendly example sentences in dictionaries in "The Usability of Example Sentences Found in English Dictionaries" at the 13<sup>th</sup> Japan Association of College English Teachers - English Dictionary Research Workshop in Tokyo in March 2012.

## First Foreign Languages

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Yoshiaki Shirasaki, *Professor*

### General Summery

1. I have continued educational activities for the purpose of verbal and nonverbal communication. These activities are also connected with the aim of developing moral and philosophical abilities in the field of intellectual relationships.
2. I have also studied Friedrich Schiller. This is a sort of paradigm innovation for this classic and ideological poet. Lang regarded Schiller to be a classical poet, and he was most productive writer of romantic operas. This is the reason I have continued this study.

## School of Nursing

### Basic Nursing

Sawako Haga, *Professor*  
Chieko Hanyu, *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) history of nursing, 4) support practice for patients with progressive motor dysfunction, and 5) nursing diagnosis.

#### Research Activities

Sawako Haga: Read and interpreted the medical views of Kanehiro Takaki based on *Home Hygiene and Treatment* (published in 1915). She also clarified the current status of education on physical assessment with a survey targeting teachers.

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 researched autonomic nerve activity when the elevation angle of the upper body is changed from the supine position as a bedpan is inserted, to determine which posture simplifies urination while the patient is in bed.

#### Publications

**Satake S, Okubo N, Ushiyama K, Suzuki E, Koitabashi K.** Discussion on the definition of positioning in nursing: results of a nursing report (in Japanese). *Nihon Kango Gijutsu Gakkaishi*. 2011; **10**(2): 47-56.

**Okubo N, Ushiyama K, Suzuki E, Satake S, Koitabashi K.** Discussion on the definition of positioning in nursing: results of a literature review

(in Japanese). *Nihon Kango Gijutsu Gakkaishi*. 2011; **10**(1): 121-30.

**Ito M, Okubo N, Satake S, Sakyo Y, Ohashi K, Hachigasaki R, Hishinuma M.** The usefulness of 'How to learn' teaching materials for reducing new nursing students' learning-related difficulties (in Japanese). *Seiroka Kango Gakkaishi*. 2011; **15**(2): 9-15.

## Nursing Administration

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Choko Sumiyoshi, *Professor*

Midori Nagano, *Professor*

### General Summary

Two studies were performed: “Development of indicators for assessing the quality of pressure ulcer management systems at hospitals” and “The needs of stoma outpatient services.”

### Research Activities

#### *Development of indicators for assessing the quality of pressure-ulcer management systems at hospitals*

We conducted a questionnaire survey of wound, ostomy, and continence (WOC) nurses and their supervising nurse administrators at 189 hospitals to analyze items affecting the incidence of pressure ulcers as quality indicators for pressure ulcer management systems. Structural requirements were “physicians have a sufficient knowledge of pressure ulcers,” “nurse managers have much education,” “WOC nurses have presentation skills, such as giving lectures,” and “staff nurses have awareness of prevention of compression wounds associated with bilevel positive airway pressure, tubes, etc.” These items were related to the incidence of pressure ulcers and were considered indicators of quality for personnel responsible for measures against pressure ulcers. In addition, “provision of facilities and benefits to WOC nurses by hospital directors” was thought to be an indicator of environmental maintenance for pressure ulcer management systems.

As process requirements, indicators were identified for the top managers, the responsible nurse administrators, WOC nurses, occupational and physical therapists, link nurses, and staff nurses.

Although 5 items on the Assessing Care of Vulnerable Elders Project Quality Index were found to be related to incidence, it was not reasonable to suppose that the 5 items would be used as targets and for evaluation, but they would be able to be used as minimum requirements for pressure ulcer management systems with WOC nurses belonging to hospitals.

As an outcome requirement, a sense of reward associated with work among WOC nurses was thought to be an indicator.

#### *The needs of stoma outpatient services*

The time between patient entry and exit from the examination room during outpatient ostomy care was measured, and patient identification and stoma type were recorded on a measurement form we developed. The clinic treated 346 patients a total of 1,797 times over a 600-day period. Initial treatments were longest, regardless of stoma type, whereas treatment times were significantly longer for ileostomies than for other types of stoma and displayed considerable variation. The present findings demonstrate that the

initial stoma examination and treatment took the most time, whereas the considerable variation in ileostomy treatment time suggests that a high percentage of patients have complicated care requirements. Because the national insurance system has only a single reimbursement rate for stoma care, introducing multiple reimbursement rates for stoma care may be necessary.

## Adult Nursing

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Shoko Fujino, *Professor*  
Hiroaki Murata, *Assistant Professor*

Naomi Takashima, *Professor*  
Ruka Seyama, *Assistant Professor*

### General Summary

We have studied what material students have learned about clinical practice in adult nursing. We have investigated what experiences graduates had during clinical practice while they were students. We then found how to develop the nursing process and how to communicate with patients. We plan to reflect upon these results and apply them to our education curriculum.

### Research Activities

Fujino investigated the effective touch techniques used by nurses for palliative care. She recorded interviews with 7 hospice nurses and 4 pain-control nurses about their touching of patients. The results were classified and described in 17 concepts and 11 categories. The nurses understood that touch was comforting and that they touch patients to comfort them. This touching, which Fujino calls “caring touch,” is used to strengthen the relationship between nurses and patients and to ease suffering.

Takashima reported on the relationship between patients’ quality of life and activity after gastrointestinal surgery. She also prepared an instrument to investigate and measure the stress patients experience in intensive care units in Japan.

Murata defined the experience of patients with acute respiratory failure undergoing ventilatory therapy and continued gathering data and information to develop a nursing support program. He also started research on patients with delirium.

Seyama described the experiences of patients with cancer who were treated and who made suggestions about nursing. She continued preparing the groundwork to develop a care model that includes families.

### Publications

**Kitada Y, Seyama R, Takai Y, Takei A, Kanda K.** Family nursing for end-of-life cancer patients in the general Japanese hospital ward (in Japanese). *Kitakanto Medical Journal*. 2011; **61**: 489–98.

**Hara S, Takei A, Seyama R, Takei Y, Tsunoda A.** Feelings of hope told by elderly cancer patients undertaking treatment: a qualitative study (in Japanese). *Kitakanto Medical Journal*. 2011; **61**: 509–14.

**Takei A, Seyama R, Ishida J, Kanda K.** Difficulties and coping behavior in a life of the cancer patient who experienced the oxaliplatin-induced peripheral neuropathy (in Japanese). *Kitakanto Medical Journal*. 2011; **61**: 145–52.

## Reviews and Books

**Takashima N.** Acute Nursing. In: *Clinical Nursing Generalities*. Tokyo: Igakushoin; 2012. p. 70–85.

# Gerontological Nursing

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Miyoko Sakurai, *Professor*

Junko Kusachi, *Associate Professor*

## General Summary

In the field of gerontological nursing, we have studied effective education methods, such as lectures and clinical training, from the perspective of the quality of life of elderly persons.

## Research Activities

The main research activities in our department have focused on edema in the lower limbs of elderly persons.

Sakurai has been investigating the psychological conflicts of sons caring for elderly parents with dementia.

Kusachi has been investigating the attitudes in difficult situations caused a psychological conflict for caregivers in facilities for the elderly.

# Mental Health and Psychiatric Nursing

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Masashi Kawano, *Professor*

Junko Ishikawa, *Assistant Professor*

## General Summary

### *Education and Research*

In education, we continued revising collaborative lectures, laboratory activities, and practical training for the fourth year. For freshmen, we gave lectures about the concepts and current issues of mental health. For sophomores, we gave lectures about working with psychiatrists and about psychiatric diagnosis and nursing care for patients with psychiatric illnesses. We invited guest speakers who were mentally ill persons from Kagayakikai to help students understand the features of mental illness. For juniors, education involved mainly laboratories about communication skills, interpersonal relationships, and the nursing process using DVDs produced by our department, with Kichijoji Hospital used for the subsequent practical training. The final examination we gave was not a

paper test but a practical examination involving role play. For junior students we trained at Kichijoji Hospital and on Ward 11E at The Jikei University Hospital. For senior year comprehensive practical training, we redefined the goals and objectives for deeper understanding of and use of skills for patients with acute psychiatric illnesses at Tokyo Musashino Hospital.

In research, we finished a study comparing psychiatric health care for patients in rural areas between Japan and Thailand and a study of child and adolescent psychiatric care in Itabashi, Toshima, and Nerima Wards of Tokyo. We continued our research on communication skills needed for undergraduate and graduate school in nursing. In particular, we focused on the human caring approach and discourse analysis. Other studies examined the utilization of recovery clubs by alcoholics and analyzed persons recovering from depression.

### *Evaluation*

The department is in the fourth year of redefining the contents of education. We have received feedback from students of each class and practical training session. This feedback indicated that the goals of classes and practical training were achieved. Feedbacks made some effective change. We will need one more year to revise the entire contents of education. Producing and using a DVD contributed to classroom teaching and practical training and strengthened the relation between the hospital and the School of Nursing. It is important to continue applying the evaluations from students to improve lectures, laboratories, and practical training. Next year we will use simulated patients for the final examination of junior students to enhance their clinical skills.

Research activity is needed to maintain a psychiatric mental health-oriented approach. Applying research results to education is essential.

### **Publications**

**Kawano M, Katayama N, Ishikawa J.** To discuss one case utilizing wellness approach toward recovery of the client (in Japanese). *Nihon Psychotherapy Gakkai Zasshi*. 2011; **12**: 69–76.

**Ando M, Kawano M.** Analysis by strengthen model toward job training psychiatric patient's psychology and needed support (in Japanese). *Seimaria Gakuin Daigaku Kiyo*. 2012; **3**: 55–62.

## **Child Nursing**

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Kiyo Hamanaka, *Professor*

Kinu Takahashi, *Associate Professor*

### **General Summary**

The results of the research mentioned in “Cooperation of educational and medical staff to provide educational support for hospitalized children” have been publicized, which we consider a significant achievement. We have also been conducting further studies the result of which, we hope, will be applied to clinical practice. The publication of a litera-

ture review on “nursing ethics” education in nursing science universities over the last 5 years, which was mentioned in ““Nursing ethics” education in nursing science related universities: literature review over the last 5 years” is considered significant for continuing research related to ethical education.

We have greatly revised a textbook of pediatric nursing in which we have been involved, although this was not research. The revisions, which were made on the basis of current social circumstances, were considered to be of marked significance. We would like to make further revisions in response to the use of the textbook and the assessment of teachers and students.

## Research Activities

### *Cooperation of educational and medical staff to provide educational support for hospitalized children*

As in the previous year, we focused on cooperation between medicine and education and analyzed the results of a national survey regarding cooperation between medicine and education involving head and charge nurses. Some findings were presented at the chairman’s talk of the Nihon Ikuryo Association, and other findings were presented in a speech as a medical worker for the Japanese Society of Child Health Nursing. This year, we also conducted an interview survey involving school teachers who have been engaged in special needs education in the Kanto area and analyzed comments collected from educational workers.

### *“Nursing ethics” education in nursing science related universities: literature review over the last 5 years*

We performed a literature review on nursing ethics education in nursing colleges for the past 5 years, and identified future issues. This review was published in an academic journal.

## Reviews and Books

**Hamanaka K.** Cooperation of educational and medical staff to provide educational support for hospitalized children (in Japanese). *Ikuryo*. 2011; 52: 19-23.

**Hamanaka K.** Introduction: Studying pediatric nursing, Chapter1: Characteristics of children, Chapter2: What is pediatric nursing (in Japanese). In: Matsuo N, Hamanaka K, editors. *Shintaikei kangogaku zensho: shoni kangogaku gairon/shoni hoken*. 4th ed. Tokyo: Medical Friend; 2012. p. 1-6, 7-28, 29-54.

**Hamanaka K.** Chapter6-2: Health problems

associated with a chronic course, Disability and nursing, Chapter7-1: Children who undergo treatment and examination, and their families, 7-3: Children who require activity restrictions and their families, 7-5: Children with pain and their families, 7-10: Children with chronic diseases who have been treated at home and their families (in Japanese). In: Matsuo N, Hamanaka K, editors. *Shintaikei kangogaku zensho: Kenko shogai wo motsu shoni no kango*. 4th ed. Tokyo: Medical Friend; 2012. p. 426-36, 448-53, 466-73, 482-9, 536-43.

## Maternity Nursing

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Kimiko Kayashima, *Professor*

Yasuko Hososaka, *Assistant 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

#### *Sexual health problems during pregnancy, the postpartum period, and child-rearing*

A literature review was performed regarding sexual function during pregnancy, the postpartum period, and child-rearing. Decreased sexual desire was widely reported due to morning sickness and worries about miscarriage and discomfort during pregnancy and due to episiotomy pain and fatigue during the postpartum period and child-rearing. Strategies are needed to manage sexual problems arising during these periods.

#### *The effects of pessary use on daily living in patients with genital prolapse*

Factors, such as chief complaints on the first medical examination and symptoms arising during pessary use, were investigated in 91 patients who were using pessaries because of genital prolapse. Pessary use reduced the rate of the sensation of uterine descensus from 78% to 16%; however, symptoms, including leucorrhea, hemorrhage, rubefaction, adhesion and erosion of the vaginal wall, dysuria, and sexual difficulties, were also reported after pessary use, indicating the need for guidance, such as self-care methods.

#### *Microbiological, immunological, and nutritional safety of breast milk*

The microbiological and immunological safety of breast milk was investigated for various storage and thawing methods using breast milk obtained 1 month postpartum from 20 adult nursing mothers and 2 samples of artificial milk for neonates. Bacterial count is affected by the storage method. In the immunological investigation, analysis was performed with immunoglobulin A and lipase as indicators, and lipase levels were found to decrease significantly with all thawing methods.

#### *Construction of a weight-control model for pregnant women and underweight pregnant women for the prevention of low birth weight*

We performed a large-scale study at 27 obstetric facilities nationwide to investigate the effects on neonatal birth weight of the intrauterine environment, in particular, increases in the mother's body weight between prepregnancy and delivery. Pregnant women's body-mass index and weight gain during pregnancy were negatively correlated with neonatal birth weight. Underweight pregnant women maternal weight gain during pregnancy is important for fetal development.

### *Methods of cleaning neonates younger than 7 days at childbirth facilities in the Tokyo Metropolitan Area*

A questionnaire survey of 186 childbirth facilities in the Tokyo Metropolitan Area was performed regarding methods of cleaning neonates younger than 7 days. Responses obtained from 88 facilities revealed the most common cleaning methods to be “none” at 0 days old and “tub bathing” at 1 day old. Cleaning methods, such as sponge baths, were reconsidered, and tub bathing was avoided immediately after birth because of possible heat loss and skin damage. However, bathing was performed as a matter of course from 1 day old.

### Reviews and Books

**Kayashima K, Hososaka Y, Murotsu F, Nishi K.** Visit to the university of perinatal facilities and training midwives in the UK. Part 2: Midwife training college (in Japanese). *Josan Zasshi*. 2011; **65**: 1010–6.

**Kayashima K, Hososaka Y, Murotsu F, Nishi K.** Visit to the university of perinatal facilities and training midwives in the UK Part 1: Perinatal facility

(in Japanese). *Josan Zasshi*. 2011; **65**: 920–4.  
**Ishii T<sup>1</sup>, Kayashima K, Kouno Y<sup>2</sup>, Noguchi K<sup>1</sup>** (<sup>1</sup>*Iwate Pref Univ*, <sup>2</sup>*Shukutoku Univ*). Observation of the pregnant woman (in Japanese). In: Maehara S, editor. New key point series of nursing observation maternity 1. Tokyo: Chuo Hoki; 2011. p. 2–37, 80–161.

## Community Health Nursing

Junko Shimasawa, Associate Professor  
 Yoshiko Kubo, Assistant Professor

Ikuko Takahashi, Assistant Professor

### General Summary

The faculty’s research has been focused on: 1) public health nursing care to promote continued participation in community life by mentally ill patients living at home, 2) infection control in the community, 3) specific medical checkup and health guidance by occupational and public health nurses.

### Research Activities

#### *Public health nursing care for mentally ill patients living at home*

The purpose of this study was to clarify the features of assistance provided by public health nursing care to promote continued participation in community life by individuals with mentally ill patients living at home. In this study, such assistance was considered to be support that promoted continued participation in the community of the mentally ill patients in a manner suitable for that individual.

#### *Infection control in the community*

We are investigating the hand hygiene among care staff in care facilities for the elderly. Hand hygiene is a basic measure to prevent infections. The purpose of this

study was to obtain suggestions for improving hand hygiene.

*Characterizing competencies for specific medical checkup and health guidance among occupational and public-health nurses*

This study used qualitative interviews to characterize competencies regarding specific medical checkup and health guidance among occupational and public health nurses.

*Quality management system for specific health guidance*

This study was performed to support the implementation of a quality management system in a local government without adopting specific health guidance. Thus, the purpose of this study was to examine public health nurses' perception change for quality management system.

## Publications

**Yamashita K, Hatono Y, Maeno Y, Kubo Y.** A Study of the significance of the introducing a quality management system to specific health guidance in a local government: perception change public health nurses and its implications (in Japanese). *Fukuoka Kenritsu Daigaku Kangogaku Kenkyu Kiyo*. 2012; **9**: 33-42.

**Hara (Kubo) Y, Nakatani J, Kamegaya R, Ino N, Mori K, Ishihara I.** Competences of specific medical checkup and health guidance for public and occupational health nurses (in Japanese). *Nihon Kango Gakkai Ronbunshu Chiiki Kango*. 2011; **41**: 231-4.

## Home Care Nursing

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Motoko Kita, *Professor*  
Hiroko Toyama, *Assistant Professor*

Reiko Yoshida, *Assistant Professor*

## General Summary

The subjects of educational research conducted by the Department of Home Nursing included the use of mobile learning systems to help students effectively learn in home-nursing training, methods to improve the effects of exercise-based home-nursing classes, and other themes of interest to teachers.

## Research Activities

*Basic study II regarding the utilization of a mobile learning system in practical training*

As reported last year, we have used mobile learning to create better learning environments for the practical training of students,. This year, we provided portable computers to individual students so that they could collect necessary information at any time and created environments to promote computer use during training. As a result, the students were able collect necessary information at any time through access to the Internet and to prepare records, facilitating the effective use of training time. These results suggest the

usefulness of mobile learning. In the future, contents should be developed regarding specialized matters about which sufficient information cannot be obtained on the Internet.

*A study of the methods to improve the effects of exercise-based lessons in home nursing*

Conventional exercise-based classes in home nursing have centered on role-playing using case examples to encourage students to develop their practical skills.

Along with an increase in lesson hours for home nursing related to the curriculum revision last year, we continuously assessed lessons with respect to lesson construction, cases, and students' learning methods to review more effective practical lessons on home nursing. The results of students' assessment of practical lessons on home nursing were favorable through the 2-year lesson assessment; although practical lessons using cases were considered difficult, the desire to accomplish these lessons was realized, and subjective learning was achieved. Concrete suggestions to conduct practical lessons using cases in the future could be obtained.

*Investigation of the usefulness of family support using the family life stability scale*

In this study, we investigated whether the Family Life Stability Scale (FLSS), a instrument for measure life stability in families continuing home nursing, could be used to evaluate the outcome of support for the families of elderly persons requiring nursing. The correlation between the families' FLSS scores and interview-based qualitative data was favorable. The results from the FLSS inferior scale scores regarding families' strong and weak points and support required were consistent with those of the comparison of the contents of nurses' assessment. Therefore, the FLSS may be useful for evaluating the family's actual state and examining the validity of support.

*The effects of a narrative intervention approach on the anticipatory grief felt by family members who provide care until the death of a terminally ill patient*

Family members who provide nursing care at home for a dying patient sometimes have a feeling of anticipatory grief. We studied the families of terminally ill patients (expected to die within 6 months) to examine the effects of a narrative approach (an intervention) on anticipatory grief, including changes in its severity and characteristics. This study was continued from last year and is still in progress.

*Inspection and evaluation*

More effective educational methods and environments must be continuously developed to improve students' nursing ability. All studies by each teacher involve important themes in the field of home nursing. These studies should be continued, supported by colleagues of this field.

## Reviews and Books

**Kita M.** Case study for clinical practice; Research design and the constructional element (in Japanese). *Kango Jissen no Kagaku*. 2011; **36**(4): 58-61.

**Kita M.** Case study for clinical practice; Role of the theory and quality assessment for the research design (in Japanese). *Kango Jissen no Kagaku*. 2011; **36**(5): 60-3.

**Kita M.** Case study for clinical practice; 4 types of the case study design (in Japanese). *Kango Jissen no Kagaku*. 2011; **36**(6): 54-8.

**Kita M.** Case study for clinical practice; Preparation for case study research (in Japanese). *Kango Jissen no Kagaku*. 2011; **36**(8): 62-5.

**Kita M, Ito K, Noguchi M, Akiyama M, Ogane H.** Reporting case study (in Japanese). *Kango*

*Jissen no Kagaku*. 2012; **37**(1): 54-9.

**Kita M.** Aging change of the mental, neural, and psychological functions (in Japanese). In: Mizutani N, Mizuno T, Takayama S, Takasaki K, editors. *Gerontological Nursing*. revised ed. Tokyo: Nippon Kango Kyokai Syuppankai; 2011. p. 84-9.

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