# Contents

# Introduction

It is my great pleasure to publish *Research Activities 2008,* which is a report on the scientific and educational activities at The Jikei University School of Medicine in 2008. 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 Center at The Jikei University School of Medicine in 2008. 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 staff of our university. Research is an important activity of a university. As President of The Jikei University School of Medicine, I have been encouraging our staff to perform research works that, in particular, support medical treatment and disease prevention. The staff members of clinical departments are working in the attached hospitals as physicians and have little time to spend in laboratories. However, our attached hospitals have 2600 beds, and 7200 outpatients are seen each day. Therefore, we are in a good position to accumulate clinical data, which can be used for clinical studies, including clinical trials. I would like to encourage our staff to publish papers regarding clinical studies as well as basic research works. The results of research will help improve both diagnosis and treatment.

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

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

Satoshi Kurihara
President
The Jikei University School of Medicine

January 14, 2010

# Continuing Medical Education Center
# The Continuing Medical Education Committee

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 doctors who have been approved by The Jikei CME Center. Members are allowed to use the facilities (video, library) of the Center and other facilities (medical library, medical museum) of the university. A telephone service is available at all times. Members may also attend or participate in summer and monthly seminars sponsored by the Center and in scientific meetings and conferences held by the department.

## Activities

1. Registered members: 245 (as of April 1, 2009)
   Members using the Center: 169/year
   Telephone service: 117 cases
2. The 29th summer seminar was held on August 2, 2008. Seventy-four persons participated.
3. Monthly seminars were held on the second Saturday afternoons of the month in April, May, June, July, September, November, February, and March. Twenty-five to 30 persons attended each seminar.
4. The "CME Center News" is mailed monthly to registered members.

# Center for Medical Education

Osamu Fukushima, *Director and Professor*
Mariko Itsubo, *Professor*
Hisashi Onoue, *Associate Professor*
Masato Matsushima, *Associate Professor*
Hideki Sasaki, *Associate Professor*
Sugino Oishi, *Associate Professor*
Yoshio Ishibashi, *Assistant Professor*
Toshikazu Sakuyama, *Assistant Professor*
Naofumi Kimura, *Professor*
Tetsuya Kawamura, *Associate Professor*
Hideaki Kashiwagi, *Associate Professor*
Kazunori Utsunomiya, *Associate Professor*
Machiko Hirao, *Associate Professor*
Nobuyuki Furutani, *Assisant Professor*
Hiroyuki Takahashi, *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: 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, management of faculty development and education seminars; and the implementation of tutorials, objective structured clinical examinations (OSCEs), and community-based medical education programs in undergraduate curricula. However, many improvements are needed in undergraduate medical and nursing education, postgraduate clinical training programs, and continuing professional development for health-care workers. In 2005, the Office of Educational Development was reorganized as the Center for Medical Education. The Center consists of the Office of Medical Education, the Office of Nursing Education, the Office of Postgraduate Clinical Training, and the Office of Educational Development. Furthermore, the secretariat was set up in the Center in April 2006. The Office of Medical Education contributed to revisions of the undergraduate curriculum, to implementation of OSCEs in years 4 and 5, and to faculty development programs (writing multiple-choice questions, and rater training for OSCEs). The Office of Nursing Education contributed to faculty development programs for nursing teachers (physical assessment training). The Office of Postgraduate Clinical Training contributed to the management of the residency program as active members of the faculty, the revision of Clinicopathological Conference (CPC) for doctors in postgraduate years 1 and 2, and implementation of faculty development programs for attending doctors belonging to the 4 attached hospitals. The Office of Medical Development contributed to the establishment an e-Learning system for students and health-care providers in the community and to the implementation of several continuing learning courses (auscultation seminar and physical assessment training courses) for district nurses in the community.

## Research Activities

1. Our proposal, “Develop community-based medical education and offer continuing professional development coursed to health-care providers in the community,” was selected to receive a Supporting Grant for Interuniversity Educational Program 2008 by

MEXT. This project was a joint proposal of 4 medical schools (The Jikei University, Showa University, Toho University, and Tokyo Medical College). In the project, we promoted staff-development programs for technical staff of medical schools and for restructuring of the e-Learning system used by students of the 4 medical schools. Showa University implemented staff-development programs for administrators of student affairs, Toho University investigated interprofessional education in health-care students, and Tokyo Medical College researched community-based medical education curricula in Japan.

2. We promoted "community-based medical education for undergraduate medical and nursing students, and developing continuing professional development programs for heath-care providers in the community" with a Supporting Grant for Distinctive University Educational 2007 from MEXT. We extended family medicine practice in the 5th year of medical education from 3 days to 1 week and started primary care and selected clinical training at community hospitals for students in years 3 through 6. We provided 7 auscultation seminars to district nurses involved in the in-home-care curricula of medical and nursing schools.

3. Our program to encourage young community doctors to become clinical researchers received a Supporting Grant for Cultivating High Quality Health Care Professions According to Social Need 2007 from MEXT. Twelve young doctors learned about biological statistics, clinical epidemiology, and clinical research design via an e-Learning system and face-to-face workshops.

4. We published final reports of 2 studies, "An e-Learning system for undergraduate students and health-care providers in the community" and "Interprofessional education for undergraduate medical and nursing students," which were also supported by educational grants from MEXT.

5. Risk-management and ethics workshops at attached hospitals. We organized workshops held in April (Nishi-shimbashi), May (Aoto), June (Daisan), July (Kashiwa), August and September (Nishi-shimbashi), October (Aoto), November (Nishi-shimbashi), December (Daisan), January (Kashiwa), and February (Nishi-shimbashi).

6. Contributions to other institutions of higher education: Faculty-development lectures and workshops were held at the Tokyo Medical Association (June and November), MEXT (July), MHLW (August, December, and January), Yamagata University (September), Kanazawa Medical College (November), Kyorin University (November and January), Tokushima University (November and January), Kumamoto University (December), and Kobe University (March).

**Publications**

***Tohda S*[1]*, Nitta Y*[2]*, Fukushima O, Nara N*[1] *([1]Tokyo Med Dent Univ, [2]Common Achievement Test Organization).*** Medical education and graduate-entry program in Australia (in Japanese). *Igakukyouiku* 2008; **39:** 367–9.

***Nishigori H*[1]*, Fukushima O, Nitta Y*[2]*, Kouzu T*[3]*, Suzuki T*[4]*, Nara N*[4] *([1]Tokyo Univ, [2]Common Achievement Test Organization, [3]Tokyo Women's Univ, [4]Tokyo Med Dent Univ).*** Trends of graduate-entry programmers in the United Kingdom (in Japanese). *Igakukyouiku* 2008; **39:** 370–2.

***Fukushima O.*** A direction of improving medical education in Japan (in Japanese). *Boseieisei* **49:** 5–7.

## Reviews

***Fukushima O.*** Final report of the distinctive university educational program to support social needs supported by the MEXT. An e-learning system for undergraduate students and health-care providers in the community (in Japanese). Tokyo: 2009

***Fukushima O.*** Final report of the distinctive university educational program supported by the MEXT. The inter-professional education in undergraduate medical and nursing students (in Japanese). Tokyo: 2009.

# Department of Anatomy (Gross Anatomy and Neuroanatomy)

Yoshinori Kawai, *Professor* Mitsuyo Maeda, *Assistant Professor*

## General Summary

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

## Research Activities

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

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

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

Neurons in the cNTS are heterogeneous in cell size (50 to 450 $\mu m^2$ 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 biocytin-filled cells after whole-cell patch-clamp recording. According to the patterns

of axonal branching behaviors, cNTS cells could be classified into 2 groups: smaller cells (94.1 $\mu m^2$ in mean somal area; range, 62–120 $\mu m^2$; n=22) and larger cells (245 $\mu m^2$ in mean somal area; range, 142–411 $\mu m^2$; 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: smaller cells had larger form factors than did larger cells ($P<0.05$), and 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 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 functioning 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 networks in the cNTS. In the present study, we examined GABAergic axon terminals and investigated postnatal changes in GABAergic synaptic organizations in the rat cNTS with immunocytochemical methods 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 postnatal week 2 and that GABAergic axon terminals were reorganized at about 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 somata of smaller neurons during postnatal week 2. Such morphologic changes in axon terminals could cause changes in electrophysiological activity and might contribute to 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 required 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. Activation of *N*-methyl-D-aspartate (NMDA) receptors is implicated in this process, and blockade leads to disruption of normal circuit formation. This phenomenon has been investigated in several neural systems, including the somatosensory system, but has not been evidenced in the visceral sensory system. Ultrastructural analysis of GABAergic synapses and electrophysiological analysis of inhibitory and excitatory postsynaptic

currents of cNTS cells revealed that developmental changes in the synaptic organizations were blocked by MK-801, an NMDA-receptor antagonist, when administered from 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 cells and large cells under far stronger excitatory and inhibitory influence, respectively. Blockade by MK-801 during the critical period might leave adult neurons wired in the undifferentiated synaptic networks, possibly preventing synapse elimination and subsequent stabilization of the proper wiring.

*Glial coverage of the small cell somata in the rat nucleus of tractus solitarius during postnatal development*

Astrocytes are thought to be active participants in synaptic plasticity in the developing nervous system. Studies have suggested that the number of axosomatic synapses on the small cells of the rat cNTS decreases toward the end of postnatal week 1. Astrocytes might be involved in this phenomenon. We examined the morphological development of astrocytic processes around the small cell soma in the rat cNTS by means of light and electron microscopy. Structures positive for glial fibrillary acidic protein, glutamate-aspartate transporter, and glutamate transporter-1 within the cNTS 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 for 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 actively 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 a key role in respiratory, cardiovascular, and gastrointestinal function, contains GABAergic neurons 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, vesicular glutamate transporter 2, calbindin, and tyrosine hydroxylase (TH) 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 four subnuclei were then quantified with an unbiased dissector analysis. Calbindin-positive cells constituted subpopulations of small non-GABAergic neurons preferentially localized in the subpostrema subnucleus. TH-positive cells constituted large neurons preferentially localized in the medial subnucleus. GABAergic neurons constituted a subpopulation of small neurons, preferentially localized in the

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

### Publications

***Okada T, Tashiro Y, Kato F, Yanagawa Y[1], Obata K[1], Kawai Y ([1]RIKEN).*** Quantitative and immunohistochemical analysis of neuronal types in the mouse caudal nucleus tractus solitarius: focus on GABAergic neurons. *J Chem Neuroanat* 2008; **35:** 275–84.

***Tashiro Y, Okada T, Kawai Y.*** Postnatal development of axosomatic synapses in the rat caudal nucleus tractus solitarus. *Neurosci Res* 2008; **61(Suppl):** S160.

***Negishi Y, Kawai Y.*** Spontaneous synaptic input patterns in the rat caudal nucleus tractus solitarius. *Neurosci Res* 2008; **61(Suppl):** S216.

# Department of Anatomy (Histology and Embryology)

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

*Polypterus, a model animal for studying tetrapod evolution*
The evolutionary transition from aquatic vertebrates to terrestrial tetrapods occurred around 370 million years ago in the late Devonian period. What changes in genome functions caused such evolution and led to the acquisition of new organs remains uncertain. We have been investigating the evolution of the tetrapod body plan using a primitive actinopterygian fish, *Polypterus*, with analyses of comparative anatomy, comparative developmental biology, and comparative genomics. Because a large-scale analysis of the *Polypterus* genome has not been performed, preparations have been made to obtain the basic information. First, construction of a bacterial artificial chromosome library of *Polypterus* has been started. Genomic DNA was prepared with nucleated blood cells extracted from an adult male. The genome size was found to be 2.8 GB. This initiative is a collaborative project with Professor Asao Fujiyama of the National Institute of Genetics. In collaboration with Professor Shinichi Aizawa of the RIKEN Center for Developmental Biology, expressed sequence tag (EST) analyses have also been started using *Polypterus* embryos at stages up to gastrulation. Complementary DNAs were constructed using RNAs extracted from *Polypterus* embryos, resulting in a determination of nucleic acid sequences of 10,000 clones. Of the genes obtained, 80.1% have 1 clone. Therefore, another 10,000 clones will be sequenced. For embryos at stages later than neurulation, a complementary DNA library is now under construction, and EST analyses will be performed at the National Institute of Genetics.

*Immunohistochemical detection of intravascularly transplanted mesenchymal stem cells*
Recently some attempts have been made to treat damaged tissues and organs by transplanting functional cells differentiated from bone marrow-derived stem cells or induced pluripotent stem cells. However, revealing the behavior of the transplanted cells in a host is difficult. To trace the transplanted cells, they should have a maker that: 1) is cell-autonomous and localized in the cell; 2) is not secreted and transferred to the surrounding cells; 3) does not affect the differentiation of transplanted cells or their

blending with other cells; 4) is taken over by the descendant cells; and 5) can be detected with a light microscope.
The cells of BALB/cA$^{-CSA}$ mice have such a marker. This strain of mouse has the genetic background of the BALB/cA stain and an Hspa9 variant derived from the C3H strain. The antibody against the C3H-specific antigen, CSA, reacts with the Hspa9 variant and not with Hspa9 of BALB/cA strain.
In this study, we have investigated whether intravascularly transplanted cells derived from BALB/cA$^{-CSA}$ mice in a host mouse of the BALB/cA strain can be detected in the host tissue. Fone marrow–derived mesenchymal stem cells were obtained from femurs of BALB/cA$^{-CSA}$ mice; they proliferated and were transplanted into a BALB/cA mouse via the femoral vein. The host mouse was killed, and each organ was fixed in Bouin's solution, dehydrated, embedded in paraffin, and sectioned. The mesenchymal cells from BALB/cA$^{-CSA}$ were immunohistochemically detected on each section with an anti-CSA antibody.
A number of CSA-positive cells were detected in blood vessels of the host mouse but were not detected in any organ other than the lung. The CSA-positive cells were present in small numbers in the lung 4 days after transplantation but were not found 1 month after transplantation.
These results indicate that mesenchymal cells transplanted into the blood vessel cannot intrude into and survive in the tissue. This study suggests that the behavior of transplanted mesenchymal stem cells, induced pluripotent stem cells, and their derivative cells can be traced by using the combination of mice of the BALB/cA and BALB/cA$^{-CSA}$ strains.

*Novel method for genome-wide analysis of tissue-specific epigenetic memory*
In addition to genetic variation, epigenetics provides an added layer of phenotypic variation that might mediate the relationship between genotype and internal and external environmental factors. This finding suggests that examining the tissue-specific epigenome might help clarify tissue-specific adaptive states due to chronic exposure to internal and external environmental factors. We designed a new method to understand the cellular pathophysiology in chronic diseases by examining tissue-specific epigenomes. This method is combined with tissue-specific addition of a tag to H3.3, the histone H3 variant localized specifically in transcriptionally active regions, and chip-on-chip or chip-seq assay using specific antibodies for the tag.
This year, we constructed a conditional knock-in vector expressing tagged H3.3 dependent on Cre recombinase. We confirmed that this vector worked as expected.

*Molecular mechanism of trigeminal placode and ganglion development in the vertebrates*
The trigeminal nerve is the largest cranial nerve, containing both sensory and motor neurons responsible primarily for sensation in the face and the movements for mastication. The trigeminal ganglion comprises cells from 2 distinct origins: placode and neural crest cells. Mechanisms of its development have been well studied in the chick; however, the molecular mechanism remains unknown. We investigated the role of

fibroblast growth factor (FGF) 8 signaling and unknown genes from head ectoderm EST analysis in trigeminal nerve development. Implantation of an FGF8-soaked bead beneath the trigeminal placode of the chick suppressed expression of *Brn3a,* the earliest trigeminal placode marker, around it. Electroporation of the dominant negative type of *Sprouty2*, a repressor of FGF8 signaling, produced a similar effect. Removal of the isthmus, the source of FGF8 in the neural ectoderm, enhanced expression of *Brn3a* and of *Pax3*, another trigeminal placode marker, implying that FGF8 signaling has a negative effect on trigeminal placode induction. Genes of morphology, causable factors of diseases, and their related genes were isolated as EST clones, which may shed light upon the molecular mechanism that could bridge the gap between FGF8 signaling and known trigeminal placode markers.

*Anatomical research on diaphragm development*

The diaphragm is a muscular membrane found only in mammals which separates the thoracic cavity from the abdominal cavity. In birds, the thoracic and abdominal cavities are separated by 2 membranes, the pulmonary diaphragm and the septum obliquum, which are thought to be homologous to the mammalian diaphragm.

To investigate diaphragm development and morphogenesis processes, we observed 4 genes (*Wt1*, *Gata4*, *Slit3*, and *Raldh2*) known to be responsible for diaphragmatic hernia by means of in situ hybridization in the mouse and chicken embryo. We found that all of these genes were expressed in the primordial diaphragm of the mouse embryo. Moreover, these genes expressed in the homologous tissues of the mouse were also observed in the chicken embryo. These results suggest the membrane that separates the thoracic and abdominal cavities was acquired from the amphibian ancestor of mammals and birds. We are now preparing the Pax3 mutant mouse to study the migration pathway of myoblasts. The same experiment is being performed with 3,3′-dioctadecylindocarbocyanine iodide to label somites in the chicken embryo.

**Publications**


***Kidokoro H*[1]*, Okabe M, Tamura K (*[1]***Tohoku Univ).*** Time-lapse analysis reveals local asymmetrical changes in C-looping heart tube. *Dev Dyn* 2008; **237:** 3545–56.

***Shigetani Y, Howard S*[1]*, Guidato S*[1]*, Furushima K*[2]*, Abe T*[2]*, Itasaki N (*[1]***NIMR,** [2]***RIKEN CDB).*** Wise promotes coalescence of cells of neural crest and placode origins in the trigeminal region during head development. *Dev Biol* 2008; **319:** 346–58.

***Matsumoto K*[1]*, Miki R*[1]*, Nakayama M*[1]*, Tatsumi N, Yokouchi Y*[1] *(*[1]***Kumamoto Univ).*** Wnt9a secreted from the walls of hepatic sinusoids is essential for morphogenesis, proliferation, and glycogen accumulation of chick hepatic epithelium. *Dev Biol* 2008; **319:** 234–47.

***Tanaka K, Hashimoto H, Tachibana T, Ishikawa H, Ohki T.*** Apoptosis in the small intestine of neonatal rat using blue light-emitting diode devices and conventional halogen-quartz devices in phototherapy. *Pediatr Surg Int* 2008; **24:** 837–42.

***Yokoo T, Fukui A, Matsumoto K, Ohashi T, Sado Y (Shigei Med Res Inst), Suzuki H, Kawamura T, Okabe M, Hosoya T.*** Generation of a transplantable erythropoietin-producer derived from human mesenchymal stem cells. *Transplantation* 2008; **85:** 1654–58.

***Yanai M*[1]*, Tatsumi N, Hasunuma N*[1]*, Katsu K*[1]*, Endo F*[1]*, Yokouchi Y*[1] *(*[1]***Kumamoto Univ).*** FGF signaling segregates biliary cell-lineage from chick hepatoblasts cooperatively with BMP4 and ECM components in vitro. *Dev Dyn* 2008; **237:** 1268–83.

***Miki R*[1]*, Tatsumi N, Matsumoto K*[1]*, Yokouchi Y*[1] *(*[1]***Kumamoto Univ).*** New primary culture systems to study the differentiation and proliferation of mouse fetal hepatoblasts. *Am J Physiol*

*Gastrointest Liver Physiol* 2008; **294:** G529–39.
***Ito T, Ohi S, Tachibana T, Takahara M, Hirabayashi T, Ishikawa H, Kusakabe M, Hashimoto H.*** Development of the mucosal vascular system in the distal colon of the fetal mouse. *Anat Rec (Hoboken)* 2008; **291:** 65–73.
***Hashimoto H, Kusakabe M (Univ Tokyo), Ishikawa H.*** A novel method for three-dimensional observation of the vascular networks in the whole mouse brain. *Microsc Res Tech* 2008; **71:** 51–9.
***Yokoo T, Fukui A, Matsumoto K, Okabe M.*** Stem cells and kidney organogenesis. *Front Biosci* 2008; **13:** 2814–32.
***Iwaizumi M, Shinmura K, Mori H, Yamada H, Suzuki M, Kitayama Y, Igarashi H, Nakamura T, Suzuki H, Watanabe Y, Hishida A, Ikuma M, Sugimura H.*** Human Sgo1 downregulation leads to chromosomal instability in colorectal cancer. *Gut* 2009; **58:** 249–60.

### Reviews and Books

***Hashimoto H, Ishikawa H, Kusakabe M.*** Three dimensional observation of the vascular networks in the brain (in Japanese). *Microscopy* 2008; **43:** 229–33.
***Yokoo T, Fukui A, Matsumoto K, Okabe M.*** Stem cells and kidney organogenesis. *Front Biosci* 2008; **13:** 2814–32.

# Department of Molecular Physiology

Yoshiki Umazume, *Professor*
Shigeru Takemori, *Associate Professor*
Maki Yamaguchi, *Assistant Professor*

## General Summary

Our efforts have been concentrated on clarifying the mechanism of muscle contraction in connection with the role of intracellular water.

## Research Activities

*Activity of intracellular water*

Magnetic resonance images are produced from differential transverse relaxation of water proton nuclear magnetic resonance signals from cells and tissues. With nuclear magnetic resonance measurements, water in skeletal muscle tissue can be classified into several components characterized by the transverse relaxation rate ($T_2$). Measurements at various humidity levels allow us to directly measure the restriction energy imposed on each water component by the sarcomeric structural organization of skeletal muscle. Energy on the order of kT is stored in each water molecule surrounding the contractile proteins of the muscle. This evidence strongly supports our hypothesis that water serves as a heat sink for the contractile interaction of myoproteins.

*Protrusion of myosin heads with the increase of longitudinal strain in the sarcomere of striated muscle*

The liquid-crystalline structure of striated muscle is a great advantage for studying the linkage between structure and function with the X-ray diffraction technique. However, only the equatorial reflections had been studied in cardiac muscles because of the poor quality of the diffraction patterns. With improved specimens, we succeeded in analyzing layer lines of cardiac muscles even after the removal of actin filaments with gelsolin. With this technique we addressed the mechanism of the Frank-Staling law of the heart, which indicates that longitudinal strain strongly modulates the activation levels of cardiac muscle (known as stretch activation in skeletal muscle). Because sarcomere elongation had little effect on the lattice spacing of myofilaments at short sarcomere lengths where stretch activation is prominent in cardiac muscle, stiff connectin/titin filaments might not be involved in the mechanism of stretch activation. As another candidate, we propose that the protrusion of myosin heads from their backbone would modulate activation levels with sarcomere elongation. The intensity profiles of the layer lines in our X-ray diffraction patterns, obtained at BL-45XU at the Super Photon Ring 8 GeV (Large-scale Synchrotron Radiation Facility), allowed us to deduce head distribution at various sarcomere lengths with and without actin filaments. In cardiac muscle without actin filaments, but not in skeletal muscle, the protrusion of myosin heads from their back bone increased with sarcomere lengths. This result supports our hypothesis.

*Structure of the cardiac cell to which mutant troponin was introduced*

Several single mutations of troponin molecules have been reported to cause familial cardiomyopathies through the altered contractility of cardiac cells. From our molecular dynamics study, tropomyosin of mutant muscle fiber was predicted to be shifted compared with that of the wild type on muscle activation. To verify this prediction of our simulation, we performed an X-ray diffraction experiment in which wild-type or mutant troponin subunit T was introduced into skinned muscle specimens at beam line 15A at the Photon Factory, Tsukuba. No difference was detected in troponin reflections obtained from mutant fibers compared with that from wild-type fibers. However, the intensity of the myosin layer line that indirectly reflects the tropomyosin position was larger in the mutant fiber than in the wild-type fiber. These results indirectly support the prediction of the molecular dynamics simulation.

*Viscosity of the myofibril suspension*

Polyethyleneglycol (PEG) narrows the lattice spacing of skinned skeletal muscle sarcomeres. Because the diameter of PEG (molecular weight, 3350) seems to be several nanometers, the lattice spacing of 40 nanometers was suspected to be large enough for penetration of PEG. To determine whether PEG penetrates the sarcomere, we measured the specific gravity of myofibril suspensions from rabbit psoas muscle in the presence or absence of PEG. If PEG dose not penetrate the sarcomere, the specific gravity of the supernatant after centrifugation of the myofibril suspension would be larger than that of the myofibril suspension. Our measurements indicated that PEG diffuses into the sarcomere to reach half of the external concentration.

*Accelerometry during exercise*

With minute accelerometers attached to various parts of the body during kendo and badminton play, subtle differences in the timing of movements between skilled and unskilled players were detected. Because acceleration directly reflects acting force and because its data can be fed instantaneously back to the practicing players, accelerometry is expected to powerfully support effective training in the field.

**Publications**

***Kimura M, Takemori S.*** $CH_2$-units on (poly)-ethylene glycol radially dehydrate cytoplasm of resting skinned skeletal muscle. *J Biochem* 2008; **43:** 841–7.

***Watanabe Y*[1]*, Takemori S, Tatsumi N*[2] ***(*[1]*Seijyo Univ Economics,* [2]*Ibaragi Univ Educ).*** Development of wearable body-temperature recorders that cause minimal interference with kendo practice: The virtue of body-temperature measurements (in Japanese). *Budougaku Kenkyu* 2008; **41:** 17–23.

# Department of Cell Physiology

Satoshi Kurihara, *Professor*
Masato Konishi, *Visiting Professor*
Yoichiro Kusakari, *Assistant Professor*
Iwao Ohtsuki, *Visiting Professor*
Norio Suda, *Assistant Professor*
Norio Fukuda, *Assistant Professor*

## General Summary

The main research interests of our department are the physiology of muscle contraction and related subjects.

## Research Activities

*Intracellular regulation mechanisms of the changes in L-type $Ca^{2+}$ current induced by $\alpha_1$-adrenoceptor stimulation in the presence of a $\beta$-adrenoceptor agonist*

We have previously shown that $\alpha_1$-adrenoceptor (AR) stimulation alone potentiates L-type $Ca^{2+}$ current ($I_{Ca}$) through $\alpha_{1A}$-AR-Gq-phospholipase C (PLC)—protein kinase C (PKC)—calcium/calmodulin dependent protein kinase (CaMK) II pathway. However, the interaction of $\alpha_1$- and $\beta$-AR signaling on $I_{Ca}$ has not been fully clarified. This year, we examined the effect of $\alpha_1$-AR stimulation on L-type $Ca^{2+}$ current ($I_{Ca}$) when $\beta$-AR is stimulated in rat ventricular myocytes using a perforated patch-clamp technique. We found that $\alpha_{1A}$-AR stimulation inhibits $I_{Ca}$ in the presence of a $\beta$-AR agonist, which is opposite to the effect observed in the absence of a $\beta$-AR agonist. We also confirmed that tyrosine kinase, which might be activated by $\alpha_{1A}$-AR-Gq signaling, inhibits $\beta$-AR signaling at the receptor site (or Gs protein). Thus, $I_{Ca}$ is inhibited. This novel signaling pathway by $\alpha_{1A}$-AR stimulation could serve as a regulatory feedback mechanism when the catecholamine concentration increases under pathophysiological conditions for protecting cardiomyocytes from $Ca^{2+}$ overload.

*Intracellular regulation mechanisms of the changes in L-type $Ca^{2+}$ current induced by endothelin-1 stimulation*

Endothelin-1 (ET-1) is a potent vasoconstrictive peptide. This peptide has a direct effect on cardiomyocytes which produces a positive inotropic effect through an increase in the intracellular $Ca^{2+}$ transient. However, the intracellular mechanism of the positive inotropic effect remains unclear. Using a perforated patch-clamp and biochemical method, we found that ET-1 activated $I_{Ca}$ through the $ET_A$-receptor-Gq-PKC-CaMKII pathway, as in the case of $\alpha_{1A}$-AR signaling. We assume that ET-1 stimulation has a positive inotropic effect partly by increasing $Ca^{2+}$ entry through L-type $Ca^{2+}$ channels. The detailed molecular mechanism of the coupling of ET-1 stimulation and $Ca^{2+}$ signaling will provide new insights into the functional roles of ET-1 signaling under physiological and pathophysiological conditions in cardiac muscle.

*Intracellular mechanisms of the increase in $Ca^{2+}$ leak from ryanodine receptor by $\beta$-AR stimulation in mouse cardiac muscle*

In heart failure, chronic catecholaminergic stimulation increases diastolic $Ca^{2+}$ leakage from ryanodine receptors (RyRs) of the sarcoplasmic reticulum (SR), leading to arrhythmia and a decrease in contractility. The increased $Ca^{2+}$ leakage from the SR by $\beta$-AR stimulation might be due to the phosphorylation of RyRs through the activation of PKA or CaMKII or both. In the present study, we intended to identify which kinase activation is responsible for the enhanced $Ca^{2+}$ leakage from the SR induced by $\beta$-AR stimulation using a saponin-skinned multicellular preparation. We examined the phosphorylation levels of RyR after $\beta$-AR stimulation by using commercially available antibodies against the PKA- and CaMKII-specific phosphorylation site of RyR. We found that the increase in $Ca^{2+}$ leakage from the SR after $\beta$-AR stimulation is responsible at least for the increase in PKA-dependent RyR phosphorylation.

*Single sarcomere imaging in cardiac muscle*

Skinned cardiac fibers exhibit spontaneous oscillatory contractions (SPOCs) over a broad range of intermediate activating conditions, namely, at a pCa of 6.0 to 5.0 (Ca-SPOC), or in the presence of MgADP and Pi under relaxing conditions (ADP-SPOC). We have reported that the period of sarcomeric oscillations in fibers correlates with that of the resting heartbeat in various animal species. The present study was performed to analyze SPOCs in single cardiomyocytes of the rat. To enhance the quality of sarcomere length measurement, we used quantum dots conjugated with an antibody against $\alpha$-actinin to visualize the Z-line position during SPOC in a single sarcomere. We measured the period and amplitude of ADP- SPOC and Ca-SPOC at various sarcomere lengths and found that the period of sarcomeric oscillations is similar to that observed at the fiber level. We also measured sarcomere lengths in intact cardiomyocytes with quantum dots at various stimulation frequencies. At low frequencies (e.g., 1 Hz), the shortening and relengthening of the sarcomere during contraction simply reflected the changes in $[Ca^{2+}]_i$. However, an increase in stimulation frequency to the physiological level (3–5 Hz) caused a phase shift of shortening and relengthening due to enhancement of the relengthening speed, resulting in the waveform being similar to that observed during SPOC in skinned myocytes. These findings suggest that the intrinsic auto-oscillatory property of sarcomeres may contribute to myocardial beating *in vivo*.

*Pathophysiology of cardiac muscle in dilated cardiomyopathy*

We were supplied with a dilated cardiomyopathy model mouse by Kyushu University (knock-in of dilated cardiomyopathy troponin in mouse heart). The myocardial contractile proteins of the model mouse showed a decrease in $Ca^{2+}$ sensitivity, which was proven by measuring the pCa-tension relation of the skinned preparations.

*Pathophysiology of skeletal muscle*

1. Disuse-induced changes in fatigability in skeletal muscle

We have reported that long-term hindlimb immobilization (6 weeks) lowers the expres-

sion of the giant protein titin in the soleus muscle of the rat, resulting in a decrease in active force production via abnormal sarcomeric organization. In the present study, we investigated how immobilization affects fatigability by using Triton X-100-treated single fibers taken from the same animal model. The intracellular concentrations of inorganic phosphate ($P_i$) and $H^+$ increase in skeletal muscle during intense exercise, resulting in a fall in active force. Therefore, we examined the effects of changes in pH and the inorganic phosphate ($P_i$) concentration on maximal $Ca^{2+}$-activated force in control fibers and immobilized fibers. We found that lowering the pH from 7.0 to 6.2 decreased the maximal force in both muscles, with the magnitude significantly greater in immobilized fibers. Likewise, the inhibitory effect of $P_i$ up to 20 mM was more pronounced in immobilized fibers. These results suggest that fatigability is enhanced in immobilized muscle and that the mechanism includes a decrease in the fraction of force-generating cross-bridges coupled and the abnormal sarcomeric organization.

## Publications

***O-Uchi J, Morimoto S, Kusakari Y, Shinji H, Obata T, Hongo K, Komukai K, Kurihara S.*** Interaction of $\alpha$1-adrenoceptor subtypes with different G proteins induces opposite effects on cardiac L-type $Ca^{2+}$ channel. *Circ Res* 2008; **102:** 1378–88.

***Tanaka H[1], Suzuki H[1], Ohtsuki I, Ojima T[1] ([1]Lab Marine Biotechnol Microbiol, Grad Sch Fisheries Sci, Hokkaido Univ).*** Structure-function relationships of molluscan troponin T revealed by limited proteolysis. *BBA* 2008; **1784:** 1037–42.

## Reviews and Books

***Mizuno J[1], Arita H[2], Hanaoka K[2], Kusakari Y, Kurihara S ([1]Dept Anesthesiol, Teikyo Univ, [2]Dept Anesthesiol Pain Relief Center, JR Tokyo Gen Hosp).*** Novel assessment of intracellular calcium transient decay in cardiac muscle by curve-fitting with half-logistic function (in Japanese). *Masui* 2008; **57:** 408–9.

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***Mizuno J[1], Morita S[1], Araki J[2], Otsuji M[3], Hanaoka K[4], Kurihara S ([1]Dept Anesthesiol, Teikyo Univ, [2]Dept Cardiovasc Physiol, Okayama Univ Grad Sch Med, Dent Pharm Sci, [3]Dept Anesthesiol Fac Med, Univ Tokyo, [4]Dept Anesthesiol Pain Relief Cent, JR Tokyo Gen Hosp).*** Curve-fit with hybrid logistic function for isovolumetric left ventricular pressure curve and isometric tension of cardiac muscles (in Japanese). *Masui* 2008; **57:** 1472–84.

***Mizuno J[1], Morita S[1], Araki J[2], Otsuji M[3], Hanaoka K[4], Kurihara S ([1]Dept Anesthesiol, Teikyo Univ, [2]Dept Cardiovasc Physiol, Okayama Univ Grad Sch Med, Dent Pharm Sci, [3]Dept Anesthesiol Fac Med, Univ Tokyo, [4]Dept Anesthesiol Pain Relief Cent, JR Tokyo Gen Hosp).*** Curve-fit with hybrid logistic function for intracellular calcium transient (in Japanese). *Masui* 2009; **58:** 67–76.

# Department of Biochemistry

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

## Research Activities

*Cancer research*

1. Earlier diagnosis and therapy are the most effective means to control cancer. To establish a noninvasive and effective *in vivo* system for detecting small tumors through molecular targeting/imaging with ultrasound or infrared fluorescence, both CD147 (extracellular matrix metalloprotease inducer, basigin) and tenascin-C (TN-C), molecules that function as promoters of tumor-cell motility and metastasis and as prognostic markers, were selected as targeting molecules for cancer detection. The final goal of our study was to realize antibody-directed early cancer diagnosis with a noninvasive *in vivo* detection method. The usefulness of antibodies against human CD147 and TN-C was first determined biochemically. CD147 is expressed by almost all malignant tumors with the exception of several of those with a neurogenic nature, and the ability of antibodies to recognize CD147 was consistent and effective both *in vitro* and *in vivo*. In contrast, TN-C was detectable in only a few human cancer cell lines. Immunocytologic studies with Alexa 488—labeled rat anti-human TN-C antibodies revealed the distinct but focal and patchy distribution of intracellular TN-C fluorescence with fine intercellular deposits. Further study confirmed that TN-C expression was localized in piled-up cells rather than in flat-growing cells. Spheroid culture, a conventional 3-dimensional (3D) culture method, showed TN-C expression was higher in A431 cells than in monolayer culture. In spheroid-cultured cells TN-C was overexpressed at both the messenger (m) RNA and protein levels with simultaneous downregulation of E-cadherin and upregulation of vimentin, as in the epithelial-mesenchymal transition. Effects of microenvironmental factors on TN-C expression were further examined with a radial flow bioreactor (RFB) for a 3D culture system, which provides tissue architecture and molecular functions mimicking the *in vivo* environment. It is of interest that A431 cells growing in an RFB secreted and accumulated larger amounts of transforming growth factor (TGF) beta 1 in conditioned media than did cells in a 2-dimensional monolayer culture. A431 cells in RFB showed increased expression of the mRNAs of TGF beta 1 and TGF beta receptors 1 and 2. The A431 cells also overexpressed matrix metalloproteinase 7, a downstream molecule of the TGF beta1 signaling pathway, and showed increased release of soluble 80-kDa fragments of E-cadherin in conditioned media time-dependently, resulting in a decrease in the E-cadherin molecule at the cell surface without downregulation of its mRNA expression. This decrease in E-cadherin release promoted the liberation of beta-catenin and its nuclear partner, upregulation of lymphoid enhancer factor (LEF) 1, and accelerated secretion of Wnt protein. Additional upregulation of a transcriptional factor, high mobility group A2 (HMGA2), and the downstream protein Slug was noted. The TGF beta 1-dependent, matrix metallo-

proteinase 7-mediated upregulation of beta-catenin/LEF1 and the TGF beta 1-activated HMGA2 pathways converged to cause Slug overexpression due to the disassembly and further repression of E-cadherin. Goosecoid, a transcriptional repressor of E-cadherin, was also upregulated. Taken all the results described above together, A431 tumor cells cultured in RFB induced the epithelial-mesenchymal transition *via* the TGF beta 1 autocrine loop and overexpressed TN-C both at the mRNA and protein levels, as were molecules related to the epithelial-mesenchymal transition. Other transcriptional factors, Notch/HEY1 and nuclear factor kappa B, were also upregulated. These signals recruited the ECM-cell remodeling and angiogenetic molecules. 3D culture in an RFB is a useful tool for cancer biology, as are nude mice.

2. Glucose metabolism is another target for cancer chemotherapy. CD147 is an accessory subunit of a heteromeric lactate transporter, monocarboxylate transporter (MCT), a member of the SLC16 family of solute transporters. The MCTs transport lactate across the plasma membrane, and CD147-MCT interaction is required for MCT activity, as well for trafficking to the plasma membrane. Three-bromopyruvate (3-BrPA), a pyruvate/lactate analog, is a potent glycolytic inhibitor and candidate anticancer agent. To clarify which transporters are involved in the cellular influx of 3-BrPA, the role of MCTs was examined. The mRNAs of MCT1, MCT2, MCT4, MCT8, MCT9, and MCT14 were expressed at various levelvs in a 3-BrPA-sensitive prostate cancer cell line, PC-3. To determine which MCT molecule was involved in 3-BrPA transport, the expression of MCTs was inhibited by small interfering (si) RNAs. Resistance to 3-BrPA was found only when the PC-3 cells were transfected with the MCT1 siRNA. PC-3 cells pretreated with MCT1 inhibitors were also resistant to 3-BrPA. Furthermore, short hairpin RNA expression vectors specific for CD147 reduced the sensitivity of PC-3 cells to 3-BrPA. These results suggest that the MCT1-CD147 complex is essential for 3-BrPA uptake.

3. Resistance of tumor cells to chemotherapeutic agents is a serious problem in cancer therapy. A conjugate of doxorubicin and glutathione *via* glutaraldehyde (GSH-DXR) strongly inhibited glutathione *S*-transferase (GST) activity in rat hepatoma AH66 cells. Treatment of the cells with GSH-DXR induced apoptosis *via* activation of c-Jun N-terminal kinase (JNK) by the binding of GSH-DXR to the active center of the GSTP1-1 enzyme, including cytochrome c release from mitochondria to cytosol, caspase-3 activation, and DNA fragmentation. Through the treatment of the cells with GSH-DXR in a recent study, another possible cytotoxic mechanism after the activation of JNK was demonstrated: the induction of apoptosis *via* deamidation of B-cell lymphoma 2, extra large, followed by the translocation of Bax to mitochondria.

4. Six cell lines with resistance to epoxomicin were established. The epoxomicin-resistant cell lines are reliable tools for the therapeutic evaluation 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. This year, the relation between E-cadherin expression and proteasomal inhibition was analyzed.

*Other research*

1. Regulatory mechanisms of transcriptional co-activator with PDZ-binding motif (TAZ) protein linked to the fibroblast growth factor (FGF)/receptor signaling, which plays an essential role in ossification, were determined with osteoblast-like MC3T3-E1 cells. It was been found that FGF-2, which inhibits bone mineralization and stimulates cell proliferation, reversibly reduced TAZ protein expression in MC3T3-E1 cells. Recent studies have shown that FGF-2 and adipogenic differentiation are related and that the TAZ protein acts as a transcriptional regulator during the differentiation. When preadipocyte-like cells were cultured with FGF-2, expression of the mRNA of aP2, an adipocytic differentiation maker transcribed by peroxisomal proliferator-activated receptor (PPAR) gamma, was increased. In contrast, FGF-2 significantly decreased the expression of TAZ, which is a corepressor of PPAR gamma. These results suggest that the adipogenic differentiation involved in FGF-2 results from the reduction of the TAZ level.

2. With methods to purify and identify ubiquitinated proteins in biological materials, several ubiquitin-protein conjugates in Tris-saline soluble and Tris-saline-insoluble but 2% sodium dodecylsulfate—soluble fractions were analyzed from cadmium-exposed human proximal tubular HK-2 cells and brains of Niemann-Pick type C (NPC) disease (lipid storage disease with progressive neuronal death) model mice. Some of purified ubiquitinated proteins were determined with amino acid sequencing analysis. The HK-2 cells exposed to cadmium at a concentration of 0.07 mM (median lethal dose) showed a marked increase in levels of ubiquitinated signal transducer and activator of transcription 6. Mean levels of sodium dodecylsulfate—soluble ubiquitin-protein conjugates in cerebrums of NPC (−/−) mice (4 and 9 weeks old) were significantly higher (up to twice as high) than in wild-type or heterozygous mice.

**Publications**

***Shimada Y, Fukuda T, Aoki K, Yukawa T, Iwamuro S, Ohkawa K, Takada K.*** A protocol for immunoaffinity separation of the accumulated ubiquitin-protein conjugates solubilized with sodium dodecyl sulfate. *Anal Biochem* 2008; **377:** 77–82.

***Asakura T, Maeda K, Omi H, Matsudaira H, Ohkawa K.*** The association of deamidation of Bcl-xL and translocation of Bax to the mitochondria through activation of JNK in the induction of apoptosis by treatment with GSH-conjugated DXR. *Int J Oncol* 2008; **33:** 389–95.

# Department of Molecular Biology

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

## General Summary

Polyamines (putrescine, spermidine, spermine) are ubiquitous biomolecules necessary for cell growth. Growth stimuli induce marked increases in the polyamine content of cells, whereas a feedback mechanism prevents over-accumulation of polyamines. A protein called antizyme (AZ) plays a key role in the feedback system. AZ is induced by polyamines and blocks the cellular polyamine supply by accelerating degradation of ornithine decarboxylase, a rate-limiting enzyme for polyamine biosynthesis, and by inhibiting cellular polyamine uptake. AZ is conserved from yeasts to humans, and in mammals there are 3 AZ paralogs (AZ1-3). These AZs are further regulated by another set of regulatory proteins, antizyme inhibitors (AZINs) 1 and 2. Our goal is to clarify the significance of the complex regulatory system mediated by so many proteins and the differential roles of the regulatory proteins, as well as to develop related research tools.

## Research Activities

*Study of the physiological roles of AZ1 in knockout mice*

In homozygous AZ1-knockout mice, tissue levels of polyamines are markedly increased. We analyzed urinary polyamines in AZ1-deficient mice and found an increase in acetylated polyamines, which suggests up-regulation of tissue polyamine catabolism via acetylated derivatives. In fact, spermidine/spermine $N^1$-acetyl transferase (SSAT) activity was elevated in several tissues, including the liver and spleen, but not in the brain or liver. In addition, we found that the SSAT activity was negatively correlated with the AZ2 level in the tissues. Maximal inducible levels of AZ2 may differ among organs, and when AZ1 is absent, the lower levels of AZ2 may lead to the higher influx of polyamine into cells, resulting in the induction of SSAT.

To study a possible protective role of AZ1 against over-intake of polyamines, we administered excess spermidine (10 times standard intake) to AZ1-deficient mice and wild-type controls daily for 1 week. This treatment caused a greater weight loss in AZ1-deficient mice. After 1 week of treatment, the polyamine contents of the whole blood and liver, respectively, of AZ1-deficient mice were 3 times and 1.5 times as high as those in wild-type controls. These results suggest that the excess spermidine intake affects polyamine kinetics throughout the body, particularly in AZ1-deficient mice.

*Study of the physiological roles of AZIN1 in knockout mice*

The physiological role of AZIN1 was studied in knockout mice. We confirmed in 2 co-isogenic lines of AZIN1-knockout mice back-crossed to C57BL/6J and BALB/c that a major phenotype of AZIN1-deficient mice is partial embryonic death, although we had previously reported that AZIN1-deficiency in a mixed genetic background was

completely lethal. Biochemical analysis revealed decreases in the tissue levels of putrescine and spermidine and in urinary polyamine excretion, confirming that total polyamine synthesis is suppressed in AZIN1-knockout mice.

*Analysis of AZ2-interacting proteins*

Previously we found a specific interaction between AZ2 and cerebellar degeneration-related protein 2 (CDR2), which binds to the proto-oncoprotein c-Myc. Pull-down assays showed that AZ2 interacts directly with c-Myc. This interaction was confirmed by the expression of fluorescent protein-tagged AZ2 and c-Myc in COS-7 cells. AZ2 was distributed in both the cytoplasm and nucleus, and c-Myc was localized in the nucleus when expressed alone, but the 2 proteins were co-localized in the nucleus when co-expressed. In addition, we found that AZ2 accelerated degradation of c-Myc, just as it does for ornithine decarboxylase, in a transient expression system in 293-F cells.

We have identified 2 AZ2-interacting protein from a mouse complementary DNA library using 2-hybrid analysis, pull-down assays, and analysis of the cellular localization of fluorescent-tagged proteins. One of these protein, zinc finger HIT domain-containing protein 1 (Znhit1), interacts with the tumor suppressor p53. Using a pull-down assay with epitope-tagged proteins, we demonstrated that AZ2 and p53 competitively bind to Znhit1.

*Improvement of a bacterial system to select RNA-binding peptides targeting AZ pseudoknot RNA*

The induction of AZ expression by polyamines is mediated by a unique translational frameshift mechanism. A signal for the translational frameshifting is a pseudoknot structure on AZ messenger RNA. To understand the mechanism of pseudoknot action, we used a bacterial selection system to screen for an artificial peptide that binds to the pseudoknot structure, but we failed to find such a peptide. The selection system is based on a reporter system in which expression of the reporter gene depends on bacteriophage lambda anti-termination activity mediated by N protein that binds to an RNA sequence termed boxB. Substitution of the combination of N protein-boxB interaction with library peptide-target RNAs allows us to screen artificial RNA-binding peptides. A possible reason screening failed is the limitation in the size of heterologous RNAs that can be accommodated in the antitermination system. The effects of the lengthening of the boxB stem were therefore examined. We found that an extension of the boxB stem led to a loss of activity, which was partially reversed by extending the RNA spacer located between boxB and boxA, another element of the antitermination system. The findings will be useful for improving the screening system for novel AZ pseudoknot-binding peptides.

*Isolation and characterization of RNA aptamers against polyamines*

Rapidly growing tissues actively synthesize polyamines and contain large amounts of polyamines. Urinary polyamines have been utilized as diagnostic and prognostic indicators of malignant disorders. An enzyme immunoassay system for urinary polyamines has already been developed and commercialized. However, currently

available anti-polyamine antibodies cannot discriminate polyamine species with similar chemical structures. RNA aptamer is a functional RNA selected from a random RNA pool with an *in vitro* amplification system, called SELEX (systematic evolution of ligands by exponential enrichment), and specifically binds to the target. Aptamers are usually superior to antibodies for structural discrimination and, therefore, are potential tools for detecting biomolecules. We attempted to isolate RNA aptamers against each polyamine and develop a diagnostic system for polyamines. As a first step, SELEX was performed using spermine as a target, and 2 RNA aptamers were selected. These aptamers are specific for spermine; they do not bind to putrescine or $N^1$-acetylspermine and bind to spermidine only weakly. The 2 aptamers share 2 secondary structure regions, which may be important for their binding activities. Selection of RNA aptamers for other polyamines is underway.

**Publications**

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***Horiya S, Koh CS*[1], *Matsufuji S, Harada K*[1] (*[1]Tokyo Gakugei Univ*).** Analysis of the interaction between selected RNA-binding peptides and a target RNA containing a bulge and a GNRA-type tetraloop. *Nucleic Acids Symp Ser (Oxf)* 2008; **52:** 209–10.

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# Department of Pharmacology

Toshihiko Momiyama, *Professor*
Naofumi Kimura, *Professor*
Haruhisa Nishi, *Assistant Professor*
Seiji Hori, *Professor*
Yuji Ohno, *Assistant Professor*

## General Summary

The research interests in Department of Pharmacology include:
1) Synaptic transmission and its modulation in the central nervous system
2) Effects of new quinolones and interaction with other drugs
3) Respiratory neural activities in *Xenopus*
4) Functions of purinergic receptors
5) Effect of the urocortin family on the cardiovascular system

## Research Activities

*Synaptic transmission and modulation in the central nervous system*
Electrophysiological studies using slice patch-clamp recording were performed to analyze synaptic transmission and its modulation in the nigrostriatal and mesolimbic dopaminergic systems and in the cholinergic system in the basal forebrain. These systems are involved in various psychological functions and in such disorders as Parkinson disease and Alzheimer disease. The regulation of output from these systems to the cerebral cortex was also studied.
Another issue is the regeneration of synapses and local circuits in basal-ganglia-related disorders. Electrophysiological, morphological, and behavioral studies were performed to clarify the mechanisms and the time course of the reconstruction of synaptic organization, transmission, and functions in intact Parkinson disease model rats and cerebral ischemia model rats.

*Effects of new quinolones and interaction with other drugs*
1. Convulsant activity of new quinolones and their drug interaction with anti-inflammatory drugs
After we demonstrated that new quinolones have potent convulsant activity that could be enhanced by concurrent administration of anti-inflammatory drugs, we studied the convulsant activity of newly developed quinolones and their interactions with anti-inflammatory drugs. Intraventricular administration of garenoxacin, moxifloxacin, and sitafloxacin induced convulsions in mice dose-dependently. Their convulsant activity was not enhanced by co-administration of anti-inflammatory drugs. We also found that quinolones with an unsubstituted piperazinyl group at position 7 had stronger convulsant activity.
2. Effects of new quinolones on inflammatory responses (in collaboration with the Department of Practical Pharmacy, Keio University Faculty of Pharmacy)
We studied the effects of new quinolones on carrageenan-induced edema in rat hind paw

and on lipopolysaccharide (LPS)-induced proinflammatory cytokines in mice and mouse peritoneal macrophages. Ciprofloxacin, norfloxacin, gatifloxacin, enoxacin, and sparfloxacin reduced carrageenan-induced edema, whereas levofloxacin, tosufloxacin, and pazufloxacin did not. Ciprofloxacin, gatifloxacin, and norfloxacin decreased LPS-induced production of tumor necrosis factor (TNF) in mice (*in vivo*). These quinolones inhibited the LPS-induced TNF-$\alpha$ production in mouse peritoneal macrophages (*in vitro*). These results suggest that new quinolones are potent biological response modifiers.

3. Effects of new quinolones on mouse core temperature

We studied the effects of new quinolones on mouse core temperature. Gatifloxacin significantly decreased core temperature in mice, but levofloxacin had no effect. We are now studying the structure-activity relationship and the mechanism of hypothermic activity of new quinolones.

*Respiratory neural activities in Xenopus*

Aquatic pipid frogs, unlike other anurans, never show a sole buccal ventilation cycle but exhale air from the lung before aspirating air into the buccal cavity. Furthermore, they have inherent muscles suspected to be homologous to the mammalian diaphragm. To study why pipid frogs lack a buccal cycle, respiratory motor activities were recorded from isolated brainstem—spinal cord preparations of *Xenopus laevis*. Brainstem preparations of *Xenopus* exhibited intermittent burst complexes (lung bursts) similar to the lung ventilation cycle *in vivo*. Lung bursts spontaneously occurred in cranial nerves V, IX, and X and in the hypoglossal and third spinal nerves. Small bursts with regular cycles similar to buccal oscillation in ranid frogs were observed in cranial nerves V and X but not in the hypoglossal or third spinal nerve. These results suggest that *Xenopus* can oscillate buccal rhythms within the brainstem.

*Functions of purinergic receptors*

1. Study of purinergic receptors on human adrenocortical cells

The expression and pharmacologic function of purinergic receptors in H295R, a cell line derived from human adrenocortical cells, were studied as a model of these receptors in human adrenocortical cells. H295R cells expressed G-protein—coupled P2Y receptors, suggesting that human adrenocortical cells express multiple purinergic receptors linked to their steroidogenic function.

2. Study of a method for detecting cortisol in human saliva

The detection of cortisol in human saliva was studied using the researchers' own saliva as the cortisol source. A cortisol-detecting reagent was prepared from a mixture of sulfuric acid and ethanol for a fluorometric assay. Samples obtained under different physiological conditions (time, eating) demonstrated variable cortisol levels. The interaction of cortisol and this reagent was thus shown to be a good method for non—radioisotope-based cortisol detection.

3. Study of the mechanisms of histamine release modification by purinergic receptors in human lung mast cells

In collaboration with Dr. E.S. Schulman of Drexel University in the United States,

several plasmids with the construct of short hairpin (sh) RNA-interference for the knock-down of histidine decarboxylase and $P2Y_2$ receptors (a subtype of P2Y receptors) were synthesized. The gene knock-down systems were transfected with lentivirus to LAD2 cells, a cell line derived from human mast cells. The results suggest that purinergic receptors are related to the modification of IgE-receptor—mediated histamine release in human lung mast cells.

*Effect of the urocortin family on the cardiovascular system*

We investigated the actions of cytokines, such as angiotensin II, LPS, and TNF-$\alpha$, on the regulation of urocortin I and its related peptides in HL-1 cardiomyocytes. We concluded that such substances, which exert oxidative stresses or inflammatory stresses or both on cardiomyocytes in pathological conditions of the heart, may regulate the expression of urocortin I and urocortin II in HL-1 cardiomyocytes.

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# Department of Pathology

Hiroshi Hano, *Professor*
Masaharu Fukunaga, *Professor*
Masafumi Suzuki, *Associate Professor*
Satoru Chiba, *Assistant Professor*
Takako Kiyokawa, *Assistant Professor*
Hiroyuki Takahashi, *Assistant Professor*
Yukiko Kanetsuna, *Assistant Professor*
Yutaka Yamaguchi, *Professor*
Akihiko Sakata, *Associate Professor*
Masahiro Ikegami, *Associate Professor*
Yasushi Kikuchi, *Assistant Professor*
Takashi Nikaido, *Assistant Professor*
Koichi Nomura, *Assistant Professor*
Tohru Harada, *Assistant Professor*

## General Summary

The research projects of our department have focused on studies of the pathogenesis, histogenesis, morphogenesis, and clinical pathology of nonneoplastic and neoplastic human disease by means of light and electron microscopy, morphometry, immunohistochemistry, gene analysis, and other techniques.

*Hepatology*

On the basis of our previous studies, we have speculated that the restructuring process of the liver lobule from chronic hepatitis to liver cirrhosis represents a change from structural instability to structural stability. In other words, liver cirrhosis might represent self-organization of the liver according to the nonequilibrial thermodynamics of living systems.

Alcoholic liver cirrhosis was studied morphologically by means of the reconstruction of histologic serial sections. Changes in the arteries and lymphatic vessels were significant for the development of morphologic changes of this disease.

Morphologic changes of the liver with aging were studied by means of morphometrical analysis. The results show that the number of bile ducts decreases with aging.

*Nephrology*

We examined 287 cases of IgA nephropathy collected from hospitals nationwide. On the basis of the results of a statistical analysis of data with histologic variables, a new scale was established for grading the histologic severity of histopathological IgA nephropathy.

Histologic specimens of IgA nephropathy collected worldwide were examined by 18 nephropathologists belonging to the international committee of histological classification of IgA nephropathy. The aim was to establish a new histologic classification for IgA nephropathy.

Nineteen renal specimens obtained from pregnant women with hypertension were examined. The presence of thrombotic microangiopathy, focal glomerulosclerosis, and medullary ray damage indicated a close relation to renal vascular hypertension.

A study of transplanted kidneys revealed that medullary ray damage was caused mainly by urinary tract disorders and immmunosuppressants. Renal tissue with chronic rejection showed peritubular capillaritis and basement-membrane thickening. In addition, the number of endothelial cells positive for cavelion-1 increased. These findings

showed a close relation to the severity of chronic rejection.
We continued to histologically re-evaluate specimens of renal cell carcinoma collected in the department using revised general rules for clinical and pathological studies of renal cell carcinoma.
A total of 150 cases of hyperplasia, dysplasia, and adenoma of the renal tubules collected in the department were studied clinicopathologically.
Inflammatory pseudotumor of the kidney was examined. The pseudotumor was composed of granulomas with multinucleated giant cells and inflammatory granulation tissue with plasma cells, many of which showed immunoreactivity for IgG4. Fungi and anti-acid bacilli were also detected with special stains.

*Gastrointestinal pathology*
Risk factors for lymph node metastasis were examined in surgically resected specimens of superficial esophageal cancer. Specimens were immunohistochemically stained with D2-40. Lymphatic channels were identified. Multivariate statistical analysis showed that lymphatic invasion was closely related to lymph node metastasis.

*Gynecological pathology*
We performed a morphological study of ovarian aging involving histological investigation and microscopic measurement of 96 specimens of the ovary obtained at autopsy. The results showed that atrophy of the ovary begins at about age 30 years and progresses, especially after menopause. Atrophy of the ovary was caused mainly by volume reduction of the ovarian medulla.
A relation between ovarian atypical endometriosis and malignant ovarian tumor was examined, and cotyledenoid dissecting leiomyoma was studied clinicopathologically.

*Urogenital pathology*
We comprehensively reinvestigated the results of studies of prostatic carcinoma and published a review.

*Lung pathology*
Pathologic data obtained from 787 cases of primary lung cancer at autopsy were analyzed to examine the prevalence of lymph-node metastasis.
We are preparing to examine the epidermal growth factor receptor gene in specimens of lung cancer.

*Other*
Histologic evaluation was performed of specimens of various organs obtained from experimental animals to verify the safety of ultrasonography with phase-change nanodroplets. Four histologic stages were established according to the severity of injury induced by ultrasound.

*Oncology*
Loss of heterozygosity was studied in prostatic cancers of minimal size, of advanced

stage, and with metastasis.

Loss of heterogeneity was studied in liver cancer cells to detect candidate susceptibility inhibitor genes that play an important role in carcinogenesis, progression, and metastasis.

## Publications


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# Department of Virology

Kazuhiro Kondo, *Professor*
Minori Kamada, *Assistant Professor*
Kazuya Shimada, *Assistant Professor*

## General Summary

Human herpesvirus (HHV) 6 belongs to the human β-herpesvirus subfamily, which includes human cytomegalovirus, HHV-6, and HHV-7. The β-herpesviruses can establish a lifelong latent infection of their host and are reactivated frequently, and some evidence suggests that the molecular mechanisms of viral latency and reactivation are common among these viruses. We are studying the molecular mechanisms of latency and pathogenesis of β-herpesviruses. Additionally, we are trying to apply the viruses as tools for studying the mechanisms of fatigue and as vectors for gene therapy.
Fatigue is an important warning sign of the exhaustive state caused by severe stresses and overwork, which may also induce a variety of diseases. We have investigated the molecular mechanism of herpesvirus reactivation, which is known to be stimulated by fatigue, and identified the molecule that can induce viral reactivation during fatigue.

## Research Activities

*Molecular mechanisms and major causes of fatigue*
Fatigue is an important warning sign of the exhaustive state caused by severe stresses and overwork, which may also induce a variety of diseases. Different types of fatigue might have mechanisms in common.
For many years, lactic acid was thought to cause fatigue. However, lactic acid is now known not to cause fatigue, because lactic acid is a key substance that provides energy and because acidity caused through a build-up of lactic acid helps prevent muscle fatigue. Thus, the molecular mechanisms of fatigue have become unclear.
We have investigated the molecular mechanism of herpesvirus reactivation, which is known to be stimulated by fatigue, and identified the molecule that can induce viral reactivation during fatigue. The molecule was up-regulated more than 10 fold with fatigue induced by forced swimming or sleep deprivation.
Our study includes the novel signal transduction pathway of fatigue and its relationship with candidate fatigue-causing substances, such as cytokines, and oxidative stress.

*Identification of novel HHV-6 latent protein associated with mood disorders and molecular mechanism of fatigue due to overwork*
Mood disorders, such as depression, are frequently associated with chronic fatigue syndrome (CFS) and physiological fatigue due to overwork. Viral reactivation of HHV-6 is a possible cause of CFS, and latent HHV-6 is reactivated by overwork. We searched for peptides encoded by HHV-6 that might produce the symptoms of CFS and for the molecular mechanism of fatigue due to overwork.

We have searched for novel HHV-6 latency-associated transcripts (H6LTs) and proteins and analyzed the function and prevalence of newly identified latency-associated protein. We then studied the gene regulation of H6LTs and searched for the factor of factors that induce viral reactivation.
Results: 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 of a peptide that we named "small protein encoded by the intermediate stage transcript of HHV-6 (SITH)-1." SITH-1 significantly upregulated the intracellular calcium levels of astrocytes. Mice expressing SITH-1 showed manic behavior. A serological study showed that antibodies against SITH-1 were present in 71% of patients with CFS who had depressive symptoms, 53% of patients with depression, and 76% of patients with bipolar disorder, and 2% of healthy adults. HHV-6 reactivation during fatigue was controlled by a small upstream open reading frame regulation mechanism that was released by the kination of eukaryotic initiation factor 2 alpha, which is a stress-response mechanism in yeast.
We have identified the novel HHV-6 latent protein SITH-1, which may cause mood disorders. Furthermore, we have identified the molecular mechanism of fatigue that induces HHV-6 reactivation.
Discussion: In this study we have shown that SITH-1, a protein encoded by HHV-6 during the intermediate stage of latency, is associated with mood disorders in CFS, depression, and bipolar disorder. Moreover, a newly identified molecular mechanism of fatigue may be related to HHV-6 regulation and mood disorders.

*Latency and reactivation of β-herpesviruses*
Both HHV-6 and HHV-7 are almost universally acquired by 2 to 3 years of age. These viruses belong to the β-herpesvirus subfamily and are closely related to each other, on the basis of biological and molecular analyses. They establish life-long latency, a hallmark of herpesviruses, reactivate frequently, and are shed in saliva.
To investigate viral reactivation, we have identified the latency-associated transcripts of HHV-6 and have partially clarified the mechanism of HHV-6 reactivation. HHV-6 established latency in macrophages and kept a fairly stable intermediate stage between latency and reactivation, and the viral reactivation was induced by 2 or more factors.
HHV-6 can reactivate in immunosuppressed patients; however, the relationship between immunosuppression and the induction of reactivation is unclear. To identify the factor(s) of HHV-6 reactivation, we have studied the association between HHV-6 reactivation and work-induced fatigue in healthy adults. Immune strength is thought to deteriorate when humans are fatigued, and viral infection reflects this deterioration of immune strength. However, a relationship between fatigue and viral infection in humans has not been proven.
The DNA of HHV-6 was detected in 88% of subjects engaged in moderately intense work due to prolonged working time and other factors (the first test day). In contrast, HHV-6 DNA was detected in 23.8% of subjects immediately after a holiday (the second test day). These results show that HHV-6 is significantly reactivated on exertion. These results led to the discovery that HHV-6 DNA expressed in saliva through the

reactivation of HHV-6 is a fatigue biomarker (a biological index factor) that varies with the degree of fatigue. Accordingly, an objective method for assessing the degree of fatigue by detecting HHV-6 DNA released into saliva as a result of the reactivation of HHV-6 was developed, enabling a simple and easy method of assessing the degree of fatigue.

The amount of HHV-7 DNA was semiquantitatively measured with a double-nested polymerase chain reaction method after serial dilution of saliva. HHV-7 DNA was detected in 92% of patients with CFS. In contrast, HHV-7 DNA was detected in 50% of healthy subjects during work and in only 30% of healthy subjects at rest. The amount of HHV-7 DNA in half of the patients with CFS were 10 to 100 times greater than the mean amount in healthy subjects. These results show that HHV-7 is significantly reactivated in the chronic fatigue state that accompanies disease. These results led to the discovery that HHV-7 DNA expressed in saliva due to reactivation of HHV-7 is a fatigue biomarker (a biological index) that varies according to chronic fatigue caused by diseases or other factors. Accordingly, an objective method for assessing the degree of fatigue by detecting HHV-7 DNA released into saliva due to reactivation of HHV-7 was developed, enabling a simple and easy method of assessing the decrease in physical strength caused by chronic fatigue.

*Application of HHV-6 and HHV-7 as gene therapy vectors*

Accumulation of knowledge and various technological advances in molecular biology and molecular genetics have greatly contributed to the recent progress in life science, providing abundant information about various biological phenomena. Active research and development has continued in various fields of life science, with particular interest in the analysis of gene functions. This has led to the development of techniques and vectors for introducing isolated genes into cells and individual organisms.

Virus vectors have advantages over other known vectors in introducing a foreign gene into a cell for protein expression. The central idea underlying gene transfer with a virus vector is to introduce a foreign gene into an infected cell and transform the cell with the foreign gene under control of promoter sequences, taking advantage of the infectious capacity of the virus (productive infection, latent infection, abortive infection).

HHV-6 and HHV-7, in particular, have attracted much attention as candidate virus vectors for gene therapy, because infections with these viruses cause mild symptoms. Using herpesviruses, and HHV-6 and HHV-7 in particular, as recombinant viruses and recombinant virus vectors has advantages, which include low pathogenicity, ease of gene introduction into blood cells, such as T cells and macrophages, and introduction of relatively large genes. However, it is difficult to produce recombinant viruses and recombinant virus vectors that originate in HHV-6 or HHV-7, and no method available today can produce such viruses and vectors. One factor that makes recombination of HHV-6 and HHV-7 difficult, in addition to technical factors, is the characteristics of HHV-6 and HHV-7 genes.

We have identified the dispensable genes of HHV-6 and HHV-7 and have reported the establishment of recombinant HHV-6 and HHV-7. The dispensable locus of HHV-6 is approximately 8.4 Kbp, and that of HHV-7 is approximately 7.3 Kbp; both are useful

sites for inserting large genes. The exogenous nucleotide sequence may encode at least 1 type of substance selected from a group including bacterial artificial chromosomes, cytokine genes, ribozymes, interference RNA, immunological co-stimulator molecules, signal transduction molecules, enzymes, and chemical attractants. Furthermore, exogenous nucleotide sequences may be used for the gene therapy of mammals. Gene therapy might be used to prevent human immunodeficiency virus infection of a compromised cell caused by human immunodeficiency virus and for the immunotherapy of cancer.

## Publications

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***Kondo K.*** Chronic fatigue and viral infection. *J Ther* 2008; **90:** 458–63.

# Department of Bacteriology

Yoshimitsu Mizunoe, *Professor*
Keiko Seki, *Professor*
Hitomi Shinji, *Assistant Professor*

## General Summary

Research projects of our department have focused on: 1) the mechanism of inhibition of *Staphylococcus aureus* colonization by commensal *Staphylococcus epidermidis*; 2) the role of beta-hemolysin in the inhibition of interleukin (IL)-8 production by human umbilical endothelial cells; 3) fibronectin-mediated colonization via fibronectin binding proteins (FnBPs) in *S. aureus* infection; 4) induction of fibroblast apoptosis by intracellular *S. aureus*; 5) the mechanism of bacterial biofilm formation; and 6) molecular analysis of viable but nonculturable bacteria.

## Research Activities

*Characterization of a biofilm destruction factor of S. epidermidis*

To clarify the mechanism by which *S. epidermidis* inhibits *S. aureus* colonization, we performed an epidemiological survey and *in vitro* studies. The studies revealed the presence of 2 types of *S. epidermidis*: one that inhibits colonization of *S. aureus* (inhibitory type) and the other that does not (non-inhibitory type). The prevalence of *S. aureus* decreased significantly in the human nasal cavity in the presence of a inhibitory *S. epidermidis* strain. Inhibitory type of strain secretes a factor that inhibits *S. aureus* colonization and disrupts preexisting *S. aureus* biofilm. This factor, biofilm destruction factor, is a 27-kDa protein that belongs to a serine protease family.

*Biofilm formation of clinical isolated staphylococcus species*

Biofilm infections caused by staphylococcus species are associated with indwelling medical devices, such as intravenous catheters and prosthetic joints. Such infections are difficult to treat and often necessitate removal of the implants. A precise analysis of biofilm formation by clinically isolated staphylococci would be useful for establishing methods to prevent and treat biofilm infection. We analyzed the capacity for biofilm formation and the biofilm component *in vitro* using strains isolated from patients of The Jikei University Hospital. Biofilm formation was observed in 29.2% (7 of 24) of methicillin-sensitive *S. aureus* (MSSA) strains, 29.2% (7 of 24) of methicillin-resistant *S. aureus* (MRSA) strains, and 25.0% (7 of 28) of *S. epidermidis* strains. Among staphylococci, 2 of the 7 biofilm-forming strains were induced by NaCl, and 5 of the strains were induced by glucose. Only 1 biofilm formed by an MRSA strain was destroyed by a polysaccharide-degrading enzyme (dispersin B), but 4 biofilms formed by *S. epidermidis* were susceptible to dispersin B. On the other hand, a protein- degrading enzyme (proteinase K) destroyed 4 biofilms formed by MSSA strains and 4 biofilms formed by MRSA strains but only 2 biofilms formed by *S. epidermidis* strains. Seven of these 10

biofilms susceptible to proteinase K were destroyed by a DNA-degrading enzyme (DNase I). There were no marked differences in the frequency of biofilm formation among the clinically isolated MSSA, MRSA, and *S. epidermidis* strains.
The biofilms of *S. epidermidis* were dependent on polysaccharides; on the other hand, the biofilms of *S. aureus* were dependent on proteins. A large amount of extracellular DNA was contained in the proteinaceous biofilms.

*Restoration of culturability of starvation-stressed and low-temperature—stressed Escherichia coli O157 cells by using $H_2O_2$-degrading compounds*
Late-exponential—phase cells of *E. coli* O157 became nonculturable in sterilized distilled water microcosms at 4°C. Plate counts declined from 3 million to less than 0.1 colony-forming units (CFU)/mL in about 21 days. However, when samples of microcosms at 21 days were inoculated onto an agar medium amended with catalase or nonenzyme peroxide-degrading compounds, such as sodium pyruvate and alpha-ketoglutaric acid, plate counts increased to $10^4$ to $10^5$ CFU/ml within 48 hours. The proposed mode of action of the catalase or pyruvate is via degradation of the metabolic by-product $H_2O_2$ rather than through supplementation of a required nutrient in the recovery of nonculturable cells. Our studies were based on the assumption that the E32511/HSC strain responds to starvation and low temperature by entering a nonculturable state and that the correction of oxidative stress upon the inoculation of bacteria on agar plates promotes the recovery of nonculturable cells.

*Inhibition of endothelial IL-8 production and neutrophil transmigration by S. aureus beta-hemolysin*
Neutrophils play a crucial role in the host response to infection with *S. aureus*, which is a major human pathogen capable of causing life-threatening disease. IL-8 is a potent chemoattractant and activator of neutrophils. We have previously reported that *S. aureus* secretes a factor that suppresses IL-8 production by human endothelial cells. Here we describe our isolation of an inhibitor of IL-8 production from the supernatant and identification of it as staphylococcal beta-hemolysin. Beta-hemolysin reduced IL-8 production without cytotoxicity to endothelial cells. Pretreatment with beta-hemolysin decreased the expression of both IL-8 mRNA and protein induced by tumor necrosis factor alpha (TNF-$\alpha$). Migration of neutrophils across TNF-$\alpha$—activated endothelium was also inhibited by beta-hemolysin. In contrast, beta-hemolysin had no effect on the expression of intercellular adhesive molecule 1 in activated endothelial cells. These results show that beta-hemolysin produced by *S. aureus* interferes with inflammatory signaling in endothelial cells and may help *S. aureus* evade the host immune response.

*Contribution of FnBPs in the infection and biofilm formation of S. aureus*
*S. aureus* employs a variety of adhesins to colonize host tissues and organs by binding to extracellular matrix proteins or plasma proteins. Among them, FnBPs are thought to be the most important adhesin for interacting with host cells. As we have already found by using an *fnbA* mutant strain derived from the parental SH1000 strain, FnBPA

is important for *in vitro* and *in vivo* infections by *S. aureus* because of the effective colonization of host tissues.
To clarify the role of FnBPB in infection and the advantage for *S. aureus* to maintain such similar factors simultaneously, we constructed an *fnb*B-deficient mutant and an *fnb*A/*fnb*B double-deficient mutant from a wild-type strain, SH1000. The experiments for *in vitro* and *in vivo* infection are now under way using these mutant strains.
Furthermore, the role of these adhesins in biofilm formation is being studied. A series of experiments indicate that FnBPB likely plays a significant role in biofilm formation by *S. aureus*.

*Growth-phase—dependent interaction between fibroblasts and S. aureus*
Staphylococci of the clinically isolated OK11 strain that had been grown in brain-heart infusion broth at 37°C for 2 hours (exponential phase) and 18 hours (stationary phase) were treated with purified fibronectin to examine the participation of fibronectin in the ingestion of bacteria by L929 fibroblasts. We have shown that exponential-phase staphylococci express a large amount of FnBP on their surfaces, whereas stationary phase cells do not. The L929 fibroblasts, which have networks of fibronectin on their surfaces, ingested a large amount of bacteria in the exponential phase. The L929 fibroblasts formed microfilaments at the edges so as to ingest bacteria rapidly.

**Publications**

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# Department of Public Health and Environmental Medicine

Hiroyuki Yanagisawa, *Professor*
Toshihiko Agata, *Associate Professor*
Koh Kobayashi, *Assistant Professor*
Yuji Suzuki, *Associate Professor*
Yuichi Miyakoshi, *Assistant Professor*

## General Summary

Our major research projects in the 2008 academic year focused on: 1) the genotoxic effects of indium chloride on micronucleus induction; 2) the comet assay for detection of DNA damage; 3) DNA damage by exposure to electromagnetic fields; 4) the effects of zinc deficiency on the induction of chromosome aberrations; 5) oxygen-induced oxidative stress; 6) methods of medical informatics education and evidence-based medicine (EBM); and 7) the risk of decompression sickness.

## Research Activities

*Experimental medicine*

1. Influence on the antitumor effect of a static magnetic field

We have reported that co-exposure to a static magnetic field (SMF) increases the frequency of chromosome aberration induced by some chemicals (including antitumor agents). In this study, we investigated the antitumor effect of co-exposure to SMFs and bleomycin in L1210 leukemia-bearing mice. The increases in lifespan were 16.5% and 12.3% by exposure to a SMF (5T) and to bleomycin (5 mg/kg), respectively. The increase in lifespan increased to 34.6% by co-exposure to gradient SMF and bleomycin. Similarly, the increase in lifespan increased to 27.9% by co-exposure to a 5-T SMF and bleomycin.

2. Effect of SMFs on the induction of micronuclei and the production of 8-hydroxy-deoxyguanosine in mice bone marrow cells

We investigated the effects of an antioxidant chemical (ascorbic acid) on the co-mutagenic effects of a 5-T SMF and X-rays by means of the *in vivo* mouse bone marrow micronucleus test. Moreover, concentrations of 8-hydroxy-deoxyguanosine (8-OHdG) in bone marrow cells were determined to examine the level of oxidative stress. Increases in both the micronucleus frequency and the 8-OHdG concentration induced by co-exposure to X-rays and SMFs were observed, but these increases were inhibited by pretreatment with ascorbic acid. These results suggest that SMF exposure enhances micronuclei in mouse bone marrow cells by affecting the behavior of free radical species produced within cells.

3. Zinc deficiency and oxidative stress

We studied the effect of oxidative stress in esophageal lesions in zinc-deficient rats. Parakeratosis in the esophagus was partially suppressed by tempol, a free-radical scavenger. This result suggests that oxidative stress is partially related to parakeratosis in the esophagus of zinc-deficient rats.

4. A work of the method for analysis of 8-OHdG by gas chromatography/mass

spectrometry
8-OHdG is considered to the best variable for assessing the oxidation damage of DNA. Measurement of 8-OHdG using gas chromatography/mass spectrometry was considered.

*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 disease and intractable diseases. We also studied the methodology of medical informatics education and EBM.

2. Oxidative stress by breathing oxygen in neonates

To clarify the carcinogenicity of target organs by oxygen exposure during the neonatal period, we studied oxidative DNA damage according to 8-OHdG in newborn rats. The oxidative DNA damage was observed to increase temporarily during oxygen exposure.

3. Outpatients with diabetes were followed up for 30 years or more. The effects of age and variability of fasting plasma glucose (FPG) levels on the onset of simple diabetic retinopathy (SDR) were analyzed. Mean levels of hemoglobin A1C and FPG were independent risk factors for the onset of simple diabetic retinopathy. The risk decreased in subjects older than 42 years, perhaps because of less variability in FPG levels.

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# Department of Forensic Medicine

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

## General Summary

Our main research projects in 2008 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, the identification of war-dead remains by DNA analysis, the objective evaluation of the limits of DNA typing based on the intensity of ninhydrin treatment, age and skewed X chromosome inactivation in autopsy specimens, 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

An experimental study was performed in a murine model to examine longitudinal changes in the pathological findings of the lungs and other organs in cases of milk aspiration. Immunostaining with an anti-human $\alpha$ lactalbumin antibody showed positive reactions against the antibody over time in the lungs, kidneys, and spleen. Detection of aspirated milk in organs other than the lung would be clear evidence of intravital milk aspiration and would suggest previous or recurrent milk aspiration.

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

In general, death by drowning is diagnosed when diatoms are detected in organs other than the lungs. We speculate that bacteria are more useful markers than are plankton for the diagnosis of death by drowning. In preserved blood samples from 30 cases of freshwater drowning, specific DNA fragments of *Aeromonas sobria,* a common aquatic bacteria, were examined with the polymerase chain reaction (PCR). The DNA fragments of the bacterium were detected with nested PCR in most cases.

*DNA analysis*

1. Identification of war-dead remains with DNA analysis

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

2. 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 purple

staining after ninhydrin treatment and the limit of DNA typing as mitochondrial DNA polymorphisms, and we attempted to perform an objective evaluation to determine the target of the staining area for DNA analysis.

3. Age and skewed X chromosome inactivation in autopsy specimens

We studied the association of age and skewed X chromosome inactivation in autopsy specimens from female subjects. Two X chromosomes were differentiated with a methylation-sensitive enzyme and the human androgen receptor PCR assay. A weak correlation was found between age and the frequency of skewed X chromosome inactivation, and we attempted to apply this technique to forensic age estimation.

*Forensic toxicology*

1. Quantitative analyses of medicines and poisonous substances

Medicines and poisonous substances (abuse drugs, alcohol, carbon monoxide, cyanide, and agricultural chemicals) suspected to have caused deaths were quantitatively analyzed by gas chromatography (GC), GC/mass spectrometry (MS), and spectrum photometry in samples obtained at autopsy.

2. Qualitative and quantitative analyses of hydrogen sulfide

Analysis of hydrogen sulfide and its metabolite thiosulfate was attempted with GC/MS in cases of poisoning by inhalation of hydrogen sulfide. We detected, lethal doses of sulfide in all cases. In addition to samples of blood and urine, samples of cerebrospinal fluid were useful for analysis.

*Establishment of age estimation*

We studied the estimation of date of birth from the quantity of radioactive carbon isolated from tooth enamel. We examined the application of this technique to age estimation for forensic medicine.

**Publications**

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# Department of Tropical Medicine

Naohiro Watanabe, *Professor*
Kenji Ishiwata, *Associate Professor*
Asao Makioka, *Associate Professor*
Masahiro Kumagai, *Assistant Professor*

## General Summary

Our research concerned mast cells in malaria, immune responses to helminth infection, and the growth and differentiation of *Entamoeba*.

## Research Activities

*Malaria and mast cells*
Malaria is a severe protozoan disease of the tropics. We have studied under our own hypothesis that protection and pathogenesis in malaria depend on pericapillary mast cells through innate and acquired immunity. We have demonstrated that mast cell —derived vascular endothelial growth factor (VEGF) is responsible for protection in murine malaria. Human mast cell lines secreted VEGF upon stimulation with an extract of *Plasmodium falciparum*. Serum levels of VEGF were significantly higher in human patients with malaria than in healthy subjects. We then examined VEGF receptor (VEGFR) in murine malaria. The results indicate that VEGFR2 on vascular endothelial cells, but not VEGFR1 on macrophages, is responsible for protection. Serum levels of soluble VEGFR2 were significantly higher in patients with malaria than in healthy subjects. However, levels of soluble VEGFR1 were similar in patients and healthy subjects. These findings suggest that mast cell-derived VEGF participates in protection and pathogenesis through stimulation of VEGFR2 on vascular endothelial cells in human and murine malaria.

*Chemotactic properties of adult* Nippostrongylus brasiliensis *to mouse intestinal mucus*
Gastrointestinal nematodes are believed to be affected by intestinal mucus because they reside in mucus that covers and protects the intestinal epithelium. Their expulsion is not associated with killing by the host T cell-mediated immune systems but is likely related to the inhospitality of the mucus. Therefore, the chemotactic properties of mouse intestinal mucus on adult *N. brasiliensis* were examined. Adult worms, placed at the center of agar-coated dishes, were incubated at 37°C under 5% $CO_2$ with mucus taken from mice harboring worms (day 5-6 after infection; established-phase mucus) or during terminated expulsion (day 9-10 after infection; expelled-phase mucus). When expelled-phase mucus was placed at the center of the dish, most worms moved to the edge of the dish. In contrast, fewer worms moved to the edge when the mucus was placed at the edge of the dish. Thus, adult *N. brasiliensis* showed negative chemotaxis to expelled-phase mucus. The worms also showed negative chemotaxis to the established-phase mucus. Interestingly, the worms moved away from bile, a component of mucus, when it was placed at the center of the dish. These results suggest that worms

dislike intestinal mucus and that chemotaxis to intestinal mucus is not associated with adult *N. brasiliensis* expulsion from the small intestine in mice.

*Analysis of actin depolymerizing factor cofilin in Entamoeba*

*Entamoeba histolytica* cysts regain motility with the induction of excystation, with the amoeba passing through a small hole made in the cyst wall. Reactivation of motility and its control by actin cytoskeletal reorganization are necessary processes in excystation. This study investigated an important molecule in actin cytoskeletal reorganization: actin depolymerizing factor cofilin. *Entamoeba invadens* was used as a model for the excystation and development of *E. histolytica*. The cysts formed in an encystation medium were transferred into a trophozoite culture medium to induce excystation. A search of the *E. histolytica* and *E. invadens* genome databases indentified 1 type of cofilin for *E. histolytica* (EhCf) and 3 types for *E. invadens* (EiCf-1, Cf-2, and Cf-3). Levels of messenger RNA, measured with the real-time reverse transcriptase polymerase chain reaction, were higher in all EiCf proteins 5 hours after excystation than before the induction of excystation. Immunofluorescence staining with a rabbit anti-EiCf antibody and a mouse monoclonal anti-actin antibody showed that both cofilin proteins and the actin of trophozoites were localized immediately beneath the cell membrane. In particular, staining in pseudopodia was intense for both cofilin and actin, suggesting they are involved in amoeba motility. Cofilin and actin were also localized around the area immediately below the cell membrane in cysts. These findings demonstrate increased cofilin expression by excystation induction, cofilin colocalization with actin, and a close correlation between cofilin expression and amoeba motility.

*Entamoeba transcriptome analysis using full-length complementary DNA*

Construction of a complementary (c) DNA library is essential for transcriptome analysis. However, most conventional methods provide only incomplete cDNA lacking the 5′-terminal region. This problem was overcome by the oligo-capping method, which focuses on the cap structure of the 5′-terminal and allows construction of a full-length cDNA library including the mRNA 5′-terminal region. With this method, a full-length cDNA library was developed for *E. histolytica* and *E. invadens* trophozoites and publicized as a Full-Entamoeba database. The database contains information on 1150 and 1238 genes of *E. histolytica* and *E. invadens,* respectively. Comprehensive analysis revealed that the 5′-end untranslated region of *E. histolytica* and *E. invadens* cDNA is significantly shorter than those of other organisms by a mean of 12 and 10 base pairs each. The newly constructed full-length cDNA database complements the *Entamoeba* genome database and is useful for analyzing the transcriptional/translational regulatory mechanisms of amoeba genes.

**Publications**

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# Department of Laboratory Medicine

Satoshi Kurihara, *Professor*
Akihiro Ohnishi, *Associate Professor*
Ken Kaito, *Associate Professor*
Hiroshi Yoshida, *Associate Professor*
Tomokazu Matsuura, *Assistant Professor*
Masato Suzuki, *Professor*
Sadayori Hoshina, *Associate Professor*
Hironari Sue, *Associate Professor*
Kenichi Sugimoto, *Associate Professor*
Toshihiko Hashizume, *Assistant Professor*

## General Summary

Research projects in our department in 2008 were concerned with clinical physiology, clinical microbiology, clinical chemistry, clinical biochemistry, hematology, cardiology, clinical cell biology, and clinical psychiatry. Research achievements in each division are described below.

## Research Activities

*Clinical physiology*

We investigated whether regular exercise in the early stage of growth (5 to 20 weeks of age) prevents the over-accumulation of visceral fat mass and other risk factors for metabolic syndrome in adolescent (46 weeks of age) Otsuka Long-Evans Tokushima fatty (OLETF) rats. The rats exercised voluntarily every day using a rotary wheel. This study found that regular exercise in the early stage of growth in OLETF rats prevents the increase in body weight and the over-accumulation of the subcutaneous and visceral fat masses and also maintained lower levels of triglycerides in the serum and liver as measured at 46 weeks of age. These results suggest that regular exercise in childhood prevents the incidence of lifestyle-related diseases, such as obesity, diabetes, and hypertension, in middle age.

*Clinical microbiology*

DNA diagnosis for mycosis was performed with a basic morphological study to assist the Microbiology section of the Central Laboratory and the Rhinosinusitis division of the Department of Otolaryngology. The polymerase chain reaction (PCR) allowed a test for epidemiological studies that was faster, simpler, and less expensive than pulse field gel electrophoresis.

The treatment of infectious waste zoonoses in an experimental animal laboratory was investigated with observation and questionnaires. Guidelines for waste management involving the H1N1 influenza virus were studied with the Ministry of the Environment.

*Clinical chemistry*

1. Pharmacogenetics of hepatobiliary ATP-binding cassette transporters in hepatocellular carcinoma

An important hepatic function is the biliary and sinusoidal secretion of endogenous and exogenous substances (e.g., drug, xenobiotics), which can protect against the accumula-

tion of various mutagens and carcinogens. This function is maintained by a drug transporter system that comprises mainly ATP-binding cassette (ABC)-transporter proteins. Therefore, genetic mutations of these proteins may impair this protective system that prevents the accumulation of hazardous compounds and may lead to hepatocellular carcinoma (HCC). We analyzed the genetic polymorphisms of efflux ABC transporters in apical (canalicular) membranes and basolateral membranes in hepatocytes using DNA samples obtained from hepatitis C virus—seropositive patients with HCC (n＝58), and compared allele and haplotype frequencies with those in a group of healthy volunteers (n＝61). The risks of acquired HCC were analyzed as morbidity odds ratios. We searched for 11 single nucleotide polymorphisms (SNPs) in efflux ABC transporters (multidrug resistance—associated protein [MRP]2, bile salt export pump [BSEP], breast cancer resistance protein [BCRP], multidrug resistance [MDR]1, MRP1, and MRP3) to identify HCC susceptibility genes. No significant association was found in any single SNP during single gene testing. However, some haplotypes in the MRP1 and BSEP genes significantly differed between patients with HCC and healthy volunteers. Further, the risk of HCC was increased by the SNP combinations of 3435C＞T in MDR1 and 825T＞C in MRP1, of 3435C＞T in MDR1 and a CTCT deletion in BSEP, and of 825T＞C in MRP1 and a CTCT deletion in BSEP, because the morbidity odds ratios ranged from 3.8 to 4.5. These results suggest that combinations of several SNPs and haplotypes in efflux ABC transporters increase the risk of hepatocarcinogenesis and lead to the development of HCC in patients with chronic hepatitis. Because the precise mechanism of how ABC transporters are involved in the development of HCC remains obscure, further studies are warranted.

*Clinical biochemistry*

We investigated and clarified the following.

1. Current issues of the remnant lipoprotein assay were highlighted by a high-performance liquid chromatography (HPLC) method we developed (reported in *Lipids in Health and Disease,* 2008).
2. The lipoprotein profiles of patients undergoing hemodialysis were investigated in detail with HPLC (reported in *Annals of Clinical Biochemistry,* 2008).
3. The clinical significance of malondialdehyde-modified low-density lipoprotein, a novel clinical use of the measurement of oxidized low-density lipoprotein, was assessed (reported in presentations and special seminars at annual meetings of the Japan Circulation Society, the Japanese Society of Laboratory Medicine, and the Japanese Society of Clinical Chemistry).
4. The beneficial effects of astaxanthin on triglycerides, high-density lipoprotein, and adiponectin were clarified (reported at annual meeting of the Japanese Society of Clinical Nutrition).

*Hematology*

1. The transcription—reverse transcription concerted reaction in detecting *Mycobacterium* tuberculosis

We examined the presence of *Mycobacterium* tuberculosis in 84 samples with the

transcription—reverse transcription concerted (TRC) method, PCR, and liquid culture. The rate of agreement between the TRC method and PCR was 97.6%, and that between TRC and liquid culture was 96.4%. The discrepancy was observed in a few samples but disappeared with cryopreservation of the samples. In such samples, the detection time of the internal control was also prolonged. Therefore, we could find the false-negative phenomenon by paying attention to such prolongation. These results indicate that TRC is a satisfactory system for *Mycobacterium* tuberculosis and could be introduced to clinical use.

*Cardiology*

We have studied catheter intervention for atrial fibrillation (AF). To eliminate AF, segmental ostial catheter ablation (SOCA) has been performed to electrically isolate the pulmonary veins from the left atrium. Our purposes are to improve the method of SOCA and to increase the cure rate of AF. This year we made 3 findings related to AF.

1. Dormant reconnection of the pulmonary vein as an unusual cause of recurrent AF
2. Characteristics of the confluent inferior pulmonary vein
3. Hypoxemia in the inferior pulmonary vein is dependent on obesity.

*Clinical cell biology*

1. Immunohistochemical study of lecithin retinol acyltransferase in hepatic stellate cells in liver diseases
2. The $^{13}$C-glucose breath test for the diagnosis of insulin resistance
3. Development of a bioartificial liver using the radial-flow bioreactor
4. Ultrasonic molecular imaging for the diagnosis of small cancers
5. Plasma examination of the transforming growth factor $\beta$-activating reaction for the diagnosis of liver damage

*Clinical psychiatry*

A retrospective study is under way to clarify the clinical significance of the 6-Hz spike and wave on electroencephalography. We reported the characteristics of patients with epilepsy compelled to withdraw from society and reported on a patient with abnormal, likely epileptic, behavior during sleep. Premonitory signs and symptoms as epileptic prodromes were studied. In addition, a study was started on the first-line choice of psychotropic drugs for the treatment of epilepsies with psychiatric symptoms.

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# Department of Internal Medicine
# Division of Gastroenterology and Hepatology

Hisao Tajiri, *Professor*
Toshifumi Okusa, *Professor*
Yoshio Aizawa, *Associate Professor*
Hisato Nakajima, *Associate Professor*
Tomohiro Kato, *Assistant Professor*
Tomohisa Ishikawa, *Assistant Professor*
Mika Matsuoka, *Assistant Professor*
Yasuyuki Searashi, *Assistant Professor*
Mariko Itsubo, *Professor*
Ichiro Takagi, *Professor*
Hirokazu Nishino, *Associate Professor*
Tateki Yamane, *Assistant Professor*
Shigeo Koido, *Assistant Professor*
Atsushi Hokari, *Assistant Professor*
Kazuhiko Koike, *Assistant Professor*

## Research Activities

*Alimentary tract*

1. Two-week triple antibiotic therapy is safe and produced remission in about 70% of pateints with refractory ulcerative colitis. Mutation of the inosine triphosphate pyrophosphohydrolase gene is closely related to the adverse reactions to azathioprine/6-mercaptopurine in Japanese patients with inflammatory bowel disease.
2. The healing stage of dextran sodium sulfate—induced colitis was characterized by plasmacytoid dendritic cell (DC) subsets. These plasmacytoid DCs regulate the secretion of interleukin 10 from regulatory T cells. We conclude that colonic plasmacytoid DCs play a critical role in mucosal repair in colitis.
3. TLR8 is a X-linked inflammatory bowel disease susceptibility gene, with both common predisposing and protecting haplotypes. These associations further emphasize the importance of genetic variation in innate immunity as determinants of Crohn's disease and of ulcerative colitis.
4. Visilizumab induced apoptosis, predominantly of $CD4^+$ lamina propria T cells, whereas $CD8^+$ lamina propria T cells were largely resistant to apoptosis. Visilizumab induces apoptosis of lamina propria T lymphocytes, but not of peripheral blood T lymphocytes, from patients with ulcerative colitis through activation of caspase-3 and caspase-8—dependent pathways.
5. Assessment with the frequency scale for the symptoms of gastroesophageal reflux disease (GERD, FSSG) questionnaire identified young age, excessive intake of alcohol, and liver dysfunction as possible predictive factors. Treatment with 20 mg of rabeprazole for 4 weeks was effective for curing GERD. The results suggest that use of the FSSG questionnaire is an appropriate method for assessing the effect of treatments for GERD.
6. We found that *Helicobacter* infection increase apoptosis in the livers of C3H/He mice.

*Tumor immunology*

Local factors related to hepatocellular carcinoma (HCC) favor the generation of regulatory T cells through the inhibition of DC maturation; however, vaccination with

DC/HCC fusion cells in patients with HCC results in the recovery of DC function and the induction of antigen-specific cytotoxic T lymphocyte responses *in vitro*. DCs engineered to secrete interleukin 12 p70 delivered into sarcoma lesions promote the cross-priming of a protective $CD8^+$ T-cell response against hemoglobin beta.

*Liver*

1. Regulatory T cells in the peripheral blood of patients with hepatitis C virus (HCV) infection might play a central role in the persistence of HCV infection and on the development of HCC. Infection of cells expressing the HCV replicon with an adenovirus expressing short hairpin (sh) RNA resulted in efficient vector delivery and expression of shRNA, leading to suppression of the replicon in the cells. A 4-week course of treatment with polyethyleneglycol-coupled interferon alpha 2a can achieve a high sustained virological response rate in "interferon-sensitive" patients with a low titer genotype 2a without negatively affecting outcome.
2. We found that the Sall4, a zinc-finger transcription factor, regulated cell-fate decision in fetal hepatic stem/progenitor cells. This molecule might have clinical applications.
3. Cases of autoimmune hepatitis in which multiple liver biopsies were performed were investigated for discrepancies between histological findings and the clinical course. Regulatory T cells, a subgroup of T cells, play an important role in the pathogenesis of autoimmune hepatitis in a mouse model.
4. Diffusion-weighted imaging and media enhancement in magnetic resonance imaging were useful methods for evaluating HCC. A study of the diagnostic efficacy of these methods is underway. In addition, we attempted to clarify the carcinogenic factors associated with non-B and non-C HCC compared with those associated with viral HCC.
5. A study of intrahepatic immunological reactions showed that between hepatic retention and apoptosis, the pool of retained activated $CD8^+$T cells undergoes affinity high antigen by model mice with the portal vein injection method.
6. Validation of a food-frequency questionnaire based on food groups and indirect calorimetry was useful for estimating individual nutrient intake. We continue to study the imbalance of nutritional evaluation in chronic liver disease.
7. Results of our histological study suggest that a connective tissue growth factor might be used as a new marker of liver fibrosis.
8. We performed a study of target chemotherapy with a glutathione-doxorubicin (GSH-DXR) conjugate encapsulated in an anti-CD147 antibody—labeled liposome against carcinoma cells highly expressing CD147. This liposome-encapsulated GSH-DXR showed specific cytotoxicity against these carcinoma cells.

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# Department of Internal Medicine
# Division of Neurology

Soichiro Mochio, *Professor*
Akira Kurita, *Associate Professor*
Masahiko Suzuki, *Assistant Professor*
Hisayoshi Oka, *Associate Professor*
Kazutaka Matsui, *Assistant Professor*

## General Summary

Our research in 2008 was conducted in the following areas: 1) dysosmia in neurodegenerative diseases, 2) neuroradiological studies with nuclear medicine, 3) neurophysiological studies of diabetic polyneuropathy, and 4) basic research on motor neuron disease.

## Research Activities

*A study of dysosmia in Parkinson disease and Alzheimer disease*

In this study, dysosmia in Parkinson disease and Alzheimer disease was evaluated with a simple method using an incense stick. The rate of dysosmia was significantly higher among patients with Parkinson disease and Alzheimer disease than among healthy control subjects.

*Cliniconeuropathological evaluation of the olfactory bulb in Parkinson disease*

We investigated the incidence and extent of Lewy body—related alpha-synucleinopathy (LBAS) in the olfactory bulb of 320 consecutive patients examined at autopsy. Paraffin sections were immunostained with antibodies against phosphorylated alpha-synuclein, tyrosine hydroxylase, phosphorylated tau, and amyloid beta. LBAS was found in the central nervous systems of 102 patients and in the olfactory bulb of 85 patients. All 35 patients who had LBAS with pigmentation loss in the substantia nigra had LBAS in the olfactory bulb. LBAS in the amygdala was more strongly correlated with LBAS in the anterior olfactory nucleus than with LBAS in the olfactory bulb periphery. These results indicate a high incidence of LBAS in the aging human olfactory bulb; they also suggest that LBAS extends from the periphery to the anterior olfactory nucleus and results in clinical manifestations of Lewy body disease.

*Neuroradiological studies with nuclear medicine*

We have made significant progress on previously proposed research activities. Results of completed studies and preliminary data in support of ongoing experiment are summarized below.

1. Clinical utility of myocardial $^{123}$I-metaiodobenzylguanidine scintigraphy in parkinsonism and dementia: Myocardial $^{123}$I-metaiodobenzylguanidine scintigraphy (MIBG) is clinically useful for differentiating Lewy body disease from other neurodegenerative diseases.
2. $^{123}$I-isopropyliodoamphetamine brain single-photon emission computed tomography

study in neurodegenerative disease: By reviewing both the decrease image and the increase image, as in Two-Tail View, 3-dimensional stereotactic surface projection may provide more information on the relative distribution of blood flow and metabolism and facilitate the differential diagnosis of parkinsonian disorders using positron-emission tomography.

3. Clinical utility of myocardial $^{123}$I-MIBG scintigraphy and the Odor Stick Identification Test for the Japanese in parkinsonism and dementia

4. Direct comparison of *in vivo* accumulation of 2 amyloid imaging probes [$^{11}$C] Pittsburgh Compound-B and [$^{11}$C]BF227 in Alzheimer disease: The purpose of this study was to directly compare the characteristics of 2 amyloid probes, [$^{11}$C]Pittsburgh Compound-B (PIB) and [$^{11}$C]BF227, in the same patients. The sensitivity of [$^{11}$C] PIB for detecting amyloid beta accumulation may be much higher than that of [$^{11}$C] BF227. However, the difference in the distribution of the 2 probes presumably reflects the difference in the specificity for amyloid beta and/or the difference in the affinity to the different stage of amyloid beta aggregation in the senile plaque generation process.

*Neurophysiological studies of diabetic polyneuropathy*

The clinical utility of nerve conduction studies and neurological examination of the feet with newly established techniques was assessed in patients with diabetic polyneuropathy, in collaboration with the Department of Diabetes, Metabolism and Endocrinology. The findings of the study suggest that neurological examinations and nerve conduction studies of the feet are useful for detecting early changes in diabetic polyneuropathy.

*Elucidation of the mechanism underlying the selective vulnerability of motoneurons*

To clarify the mechanism underlying the selective vulnerability of motoneurons, we compared the membrane current responses to metabolic disturbances induced by NaCN and oxygen deprivation between neurons in the hypoglossal nucleus and the dorsal motor nucleus of the vagus nerve in brainstem slices from young rats. The results suggest that potentiation of N-methyl-D-aspartate receptor currents through facilitated glycine release by metabolic disturbance might play a role in the link between mitochondrial dysfunction and selective degeneration of motor neurons.

*Selective vulnerability of motor neurons*

We compared the effects of chemical anoxia on membrane currents and postsynaptic currents in different motor nuclei. In all nuclei, NaCN induced a persistent inward current accompanied by a marked and robust increase in action potential-independent synaptic inputs. Strychnine was more potent in attenuating the NaCN effect in cranial nerve nuclei XII and VII than in nuclei III, in which picrotoxin was more potent.

**Publications**

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# Department of Internal Medicine
# Division of Kidney and Hypertension

Tatsuo Hosoya, *Professor*
Tetsuya Kawamura, *Associate Professor*
Keitaro Yokoyama, *Assistant Professor*
Makoto Ogura, *Assistant Professor*
Masato Ikeda, *Assistant Professor*
Hiroshi Hayakawa, *Assistant Professor*
Iwao Ohno, *Professor*
Yasunori Utsunomiya, *Associate Professor*
Yasuhiro Yamamoto, *Assistant Professor*
Kazushige Hanaoka, *Assistant Professor*
Yoichi Miyazaki, *Assistant Professor*

## General Summary

Major fields of research are: 1) nephrology, 2) hypertension, and 3) uric acid metabolism. Published achievements and recent reports are summarized here.

## Research Activities

*Nephrology*

1. Glomerulonephritis

The glomeruli from patients with obesity-related glomerulopathy showed glomerulomegaly, and the glomerular number and the body-mass index were independent factors correlated with glomerular size. We demonstrated that both the nephron number and glomerular enlargement play a crucial role as compensatory mechanisms against the deterioration of renal function in chronic kidney disease. We have created inducible vascular endothelial growth factor (VEGF) transgenic mice, in which we have found several distinct glomerular phenotypes. It is suggested that Pax2-expressing human mesenchymal stem cells differentiate into the Wolffian duct by the influence of local signals in the chicken ureteric bud progenitor region.

2. Dialysis

We found that changes in bone turnover or osteoprotegerin itself affected the response of urinary phosphate excretion via fibroblast growth factor (FGF)-23 to a high-phosphate diet in osteoprotegerin knockout mice. In cultured parathyroid cells isolated from patients with secondary hyperparathyroidism, we found that L-type $Ca^{2+}$ channels play a role in the high extracellular $Ca^{2+}$–activated increase in cytoplasmic $Ca^{2+}$ concentration. We found that treatment with both peritoneal dialysis and hemodialysis is a useful way to control body fluids and that peritoneal function may be maintained for a long time. We studied acute humoral rejection and performed ABO-incompatible renal transplantation and husband-to-wife renal transplantation. In transplant glomerulopathy, glomerular expression of plasmalemmal vesicle–associated protein-1 is positively correlated with the severity of transplant glomerulopathy and proteinuria.

*Hypertension*

Insulin resistance was a significant risk factor for the deterioration of renal function in patients with hypertension and chronic kidney disease but without diabetes.

Our clinical experiment suggested that in men with untreated essential hypertension, the serum uric acid level is an independent marker of systemic arterial stiffness and microalbuminuria.

*Uric acid metabolism*

To clarify the significance of uric acid in toxemia and pregnancy-induced hypertension (PIH), the relationship between uric acid and clinical variables was investigated in patients with toxemia and PIH in our hospital. Levels of uric acid and lactate dehydrogenase (LDH) were inversely correlated with birth weight in both conditions. A significant correlation was seen between the levels of LDH and uric acid. In multiple regression analysis, only LDH was associated with birth weight in both toxemia and PIH. Some common humoral factors, such as soluble fms-like tyrosine kinase 1, might contribute to the correlation between LDH and uric acid through endothelial cell injury. In inflammatory arthritis after parathyroidectomy in patients with secondary hyperparathyroidism, pseudogout must be considered. Uric acid dynamics was investigated after renal transplantation.

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# Department of Internal Medicine
# Division of Rheumatology

Akio Yamada, *Professor*
Daitaro Kurosaka, *Associate Professor*
Isamu Kingetsu, *Assistant Professor*

## General Summary

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

## Research Activities

Clinical and experimental studies of autoimmune disease were performed.

1. Analysis of the effect of neovascularization inhibitors in animal models of rheumatoid arthritis

Several studies have reported the arthritis-inhibiting effects of neovascularization inhibitors in animal models of rheumatoid arthritis. We evaluated the effects of the neovascularization inhibitor endostatin in collagen-induced animal models of arthritis. Furthermore, we are analyzing the mechanism of the arthritis-inhibiting effects of endostatin.

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

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

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

The activation of telomerase has recently been observed in healthy cells, including normal lymphocytes. An increase in telomerase activity is associated with the activation of lymphocytes. Much attention has been paid to the role of telomerase in immunocytes. We measured telomerase activity in peripheral blood mononuclear cells obtained from patients with autoimmune diseases, especially systemic lupus erythematosus.

4. Clinical studies aimed at standardizing immunosuppressant therapy for 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 remain unclear. We have performed a clinical trial to establish a strategy for treating severe autoimmune diseases. Clinical studies aimed

at standardizing immunosuppressant therapy of autoimmune disease were performed.

### Reviews and Books

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# Department of Internal Medicine
# Division of Cardiology

Michihiro Yoshimura, *Professor*
Ikuo Taniguchi, *Professor*
Shingo Seki, *Associate Professor*
Kenichi Hongo, *Associate Professor*
Takahiro Shibata, *Assistant Professor*
Atsushi Seo, *Assistant Professor*
Kimiaki Komukai, *Assistant Professor*
Mitsuyuki Shimizu, *Professor*
Masayuki Taniguchi, *Associate Professor*
Teiichi Yamane, *Associate Professor*
Satoru Yoshida, *Assistant Professor*
Naofumi Aoyama, *Assistant Professor*
Makoto Kawai, *Assistant Professor*
Toshio Hasuda, *Assistant Professor*

## General Summary

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

## Research Activities

*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 collaborated in subanalyses in the Japanese Investigation of Kinetic Evaluation in Hypertensive Event And Remodeling Treatment (JIKEI HEART) study, whose results were reported last year, and in such studies as the Japanese Rhythm Management Trial for Atrial Fibrillation (J-RHYTHM: upstream drug therapy for atrial fibrillation associated with hypertension by means of a multicenter study, a study related to and comparative study of calcium antagonists and angiotensin receptor blocker [ARB]), the Assessment of β-Blocker Treatment in Japanese Patients with Chronic Heart Failure (J-CHF: a large-scale clinical study to establish a β-blocker treatment method in chronic heart failure), the Pitavastatin hEARTt gaiLure (PEARL) study (multicenter cooperative study to investigate the ameliorative effect of hydroxymethyl glutaryl coenzyme A reductase inhibitors on chronic heart failure), the Combination of OLMesartan and CCB or Low-Dose Diuretics in High-Risk Elderly Hypertensive Patients study (COLM study: comparing a calcium antagonist and low-dose diuretic as combination drugs for use in ARB therapy of elderly hypertension patients at risk of cardiovascular disease), and the Nationwide Gender-specific Atherosclerosis Determinants Estimation and Ischemic Cardiovascular Disease Prospective Cohort Study (NADESICO Study: multicenter cooperative prospective cohort study on sex differences in risk factors for arteriosclerotic diseases and prevention), which used computed tomography examinations of the coronary arteries.

We performed subanalyses in the JIKEI HEART Study (Mochizuki S. et al., Lancet, 2007) which were related to background factors (sex, age, ischemic heart disease, diabetes, and hyperlipidemia) and left ventricular mass index. The results of these subanalyses were presented at meetings of the Japanese Circulation Society, the Japan Geriatric

Society, the Japanese Society of Hypertension, the European Society of Cardiology, and the American College of Cardiology.

We have converted patient data, including risk factors, lesion morphology, during catheter examinations and treatment in various clinical research divisions into a database and performed a study comparing risk factors, outcome, and other variables in 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]), investigating treatment with drug-eluting stents and the diagnosis of coronary vasospasm, which is closely related to the etiology of ischemic heart disease.

With regard to heart failure, which is an extremely common form of circulatory pathology, we have been assessing data related to serum concentrations of brain natriuretic peptide, which is an index of pathology, and have been investigating standard values that will be useful in clinical practice. In addition, we are investigating the pathology of heart failure before and after hospital admission and are assessing clinical data that will serve as a new index.

We have been aggressively treating atrial fibrillation by means of catheter ablation, and in this fiscal year we treated a total of 232 patients. In addition, in clinical research we have 1) investigated the usefulness of the pulmonary vein antrum isolation procedure by voltage mapping and 2) presented papers on the suppression of re-conduction after pulmonary vein-antral isolation procedures by abolishing adenosine triphosphate re-conduction.

In research on human lipoprotein metabolism we performed a tracer experiment with stable isotopes in patients with autosomal recessive hypercholesterolemia, a rare condition everywhere in the world, in cooperation with Kanazawa University. In addition, we are analyzing the effect of ezetimibe, an inhibitor of cholesterol absorption in the small intestine, on lipoprotein metabolism.

*Basic research*

Our research activities include study at other institutions in Japan and abroad by graduate students in basic sciences and clinical sciences and the presentation of the results of many studies. In the field of arrhythmia, we have studied the effects of inflammatory cell invasion mechanisms and myocardial fibrosis on the development of atrial fibrillation in various experimental models. In the field of cardiomyocyte physiology, we have investigated the physiological and pathophysiological regulatory mechanisms of myocardial contraction and relaxation by means of both molecular biologic techniques and physiologic 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 effects of $\beta$-receptor stimulation in sarcoplasmic reticulum function, and cardiomyocyte intracellular Ca kinetics in mice with dilated cardiomyopathy due to 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.

## Publications


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# Department of Internal Medicine
# Division of Diabetes, Metabolism and Endocrinology

Naoko Tajima, *Professor and Chairperson*
Junichi Yokoyama, *Professor*
Takashi Sasaki, *Professor*
Kuninobu Yokota, *Associate Professor*
Hideaki Kurata, *Associate Professor*
Tamotsu Yokota, *Assistant Professor*
Yoichi Sakamoto, *Professor*
Kazunori Utsunomiya, *Professor*
Katsuyoshi Tojo, *Professor*
Yutaka Mori, *Associate Professor*
Masami Nemoto, *Associate Professor*
Rimei Nishimura, *Assistant Professor*

## General Summary

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

## Research Activities

*Epidemiology and evidence-based medicine*

A nationwide epidemiologic study of mortality in approximately 1,500 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 are being performed.

*Molecular diabetology and regenerative medicine*

Increased cellular injury and reduced capacity in regeneration are novel therapeutic targets in diabetes. Our study group has succeeded in direct in vivo transfer of the CKD4 gene, a cell-cycle regulator. We have confirmed that regulated proliferation of mature beta cells results in restoration of glucose metabolism in diabetic mice. The result was presented in the 44$^{th}$ annual meeting of the European Association for the Study of Diabetes.

Our study group also has focused on the genetic epidemiology of the development of diabetes and its complications. This year a protocol was prepared for a new prospective study in a specific cohort with diabetes-susceptibility genes that have been identified with the genome-wide screening.

*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 (an inhibitor of dipeptidyl peptidase IV) on insulin resistance were investigated.

*Dietary therapy*

A highly monounsaturated enteral formula suppressed postprandial hyperglycemia without exaggerated insulin secretion better than did a high-carbohydrate enteral formula in patients with type 2 diabetes mellitus and in healthy subjects. Continuous glucose monitoring showed that a highly monounsaturated eternal formula significantly suppressed postprandial hyperglycemia and markedly reduced the 24-hour glycemic variations in patients with type 2 diabetes receiving tube feeding.

*Diabetic vascular complications*

Research has focused on the pathogenesis and treatment of diabetic vascular complications. Clinical studies have examined dietary therapy for type 2 diabetes. Experimental studies using vascular endothelial cells, mesangial cells, neural cells, and retinal pericytes have investigated the role of Krüppel-like factor 2, Rho/Rho-kinase, and the peroxisomal proliferator-activated receptor $\alpha$-mediated signaling pathway in the pathogenesis of diabetic vascular complications and have provided evidence that these molecules are potential pharmacological targets in the treatment of diabetic vascular injury.

*Endocrinology*

The effect of azelnidipine, a unique dihydropyridine calcium-channel blocker, on aldosterone synthesis was evaluated in NCI-H295R, a human adrenocortical cell line.
The involvement of corticotropin-releasing hormone receptor signaling against vascular inflammatory stress was evaluated using human aortic endothelial cells.
The potential role of cardiovascular stress in the regulation of the urocortin-corticotropin-releasing hormone receptor system was evaluated in HL-1 cardiomyocytes.
Immunohisochemical analysis of resected tumor specimens obtained from patients with Cushing disease and subclinical Cushing disease was performed to evaluate the expression of 11$\beta$-hydroxysteroid dehydrogenase types 1 and 2 and glucocorticoid receptor.
DNA molecular typing was performed in 2 cases of familial Graves disease associated with type 1 diabetes with strikingly homologous clinical features.

**Publications**

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# Department of Internal Medicine
# Division of Clinical Oncology/Hematology

Keisuke Aiba, *Professor*
Fumi Mizorogi, *Professor*
Daisuke Inoue, *Associate Profesor*
Toshikazu Sakuyama, *Assistant Professor*
Osamu Asai, *Assistant Professor*
Shuichi Masuoka, *Assistant Professor*
Shingo Yano, *Assistant Professor*
Tadashi Kobayashi, *Professor*
Noriko Usui, *Associate Profesor*
Toshio Katayama, *Assistant Professor*
Takaki Shimada, *Assistant Professor*
Nobuaki Dobashi, *Assistant Professor*
Yoshikazu Nishiwaki, *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 hematological malignancies and solid tumors, 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 2008.

## Research Activities

*Leukemias*

Many patients with previously untreated hematological disorders have been referred to our department. The disorders in 2007 included acute myeloid leukemia (AML), 11 cases; acute promyelocytic leukemia (APL), 1 case; acute lymphoblastic leukemia (ALL), 8 cases; chronic myeloid leukemia (CML), 9 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 2008 were as follows: 1) AML/MDS-HR CS-7 study: newly diagnosed AML, refractory anemia with excess blasts II, all case registration cohort study; 2) AML-201: very late antigen 4 study (prognosis evaluation study); 3) AML-206 DNR assigned group (relapse and refractory AML: phase I); 4) APL-204 (phase III); 5) APL-205R (relapsed and refractory APL: phase II); and 6) ALL-202 (phase III). We also performed several collaborative group studies and pilot studies: 1) Aged Double-7 (newly diagnosed aged AML: phase II); 2) VEGA study (MDS: phase II); 3) a nilotinib study (refractory CML: phase I/II), and 4) a dasatinib study (refractory CML: phase I/II).

*Lymphomas*

In 2008 we registered 83 patients with newly diagnosed non-Hodgkin's lymphoma and 4 patients with Hodgkin's lymphoma. We have performed clinical trials as a member of the Lymphoma Study Group of the Japan Clinical Oncology Group (JCOG-LSG). The studies JCOG0211-DI (newly diagnosed natural killer/T lymphoma: phase I/II)

and JCOG0203-MF (newly diagnosed follicular lymphoma: phase III) were pivotal protocol studies in 2007. Other cooperative studies examined biweekly rituximab, etoposide, prednisone, vincristine, and hydroxydaunorubicin (R-EPOCH: relapsed and refractory B-cell lymphoma: phase II); pirarubicin, cyclophosphamide, vincristine, and prednisolone (THP-COP: newly diagnosed T-cell lymphoma: phase II); and enzastaurin (non-Hodgkin's lymphoma: phase III, double-blind).
Enzastaurin is a novel drug targeting protein kinase C$\beta$ that has been extensively studied throughout the world, including the United States, the European Union, and Japan.

*Myeloma*
We registered 6 patients with newly diagnosed multiple myeloma in 2008. We completed our original pilot study evaluating a combination of thalidomide and dexamethasone in 2007. Bortezomib, a novel proteasome inhibitor, became available in 2007, and we have used it to treat patients who have refractory myeloma.

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

*Solid tumors*
Many patients with solid cancers have been referred to our department from related divisions or departments in our hospital. In 2006, the disorders included breast cancer, 97 cases; esophageal cancer, 24 cases; gastric cancer, 21 cases; colorectal cancer, 62 cases; and other cancers, 38 cases. Several of our studies are in progress throughout our university hospital with related divisions or departments, seeking improved therapeutic outcomes. 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. The standard treatment for operable, locally advanced esophageal cancer has been altered, resulting in the use of chemoradiation therapy rather than surgical resection. We, therefore, have been investigating a combined modality therapy of radiation and chemotherapy with low dose-cisplatin and 24 hours' continuous infusion of 5-FU for such patients since 2002.The results will be reported next year. For patients with advanced gastric cancer, combination chemotherapy with S-1 and cisplatin has been performed. Our first-line chemotherapy regimens for patients with advanced colorectal cancer are folinic acid, 5-FU, and oxaliplatin (FOLFOX), and folinic acid, 5-FU, and irinotecan (FOLFIRI). Since antibodies against vascular endothelial growth factor became available in 2007, therapies combining them with FOLFOX or FOLFILI have been performed.

*Palliative care*

The mission of the Palliative Care Team for Cancer Pain Purposes 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 new 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 hematological malignancies and solid cancers. The structural differences between M protein produced by myeloma cells and that from monoclonal gammmopathy of undetermined significance have been examined, and the function of ATP-binding cassette transporters in cancer chemotherapy has also been studied in collaboration with Keio University Department of Pharmacy.

**Publications**

***Kitajima K, Kobayashi S, Shiba H, Uwagawa T, Ishida Y, Aiba K, Kawakami M, Yanaga K.*** Successful treatment of advanced gallbladder cancer with an anticancer drug S-1: assessment based on intratumoral gene. *Int J Clin Oncol* 2008; **13:** 545–51.

***Konishi T, Sasaki T, Aiba K, Fukutomi T, Kakegawa K, Okubo T.*** Importance of the primary physician for pain management in patients with recurrent advanced cancer:a questionnaire survey. *Gan To Kagaku Ryoho* 2009; **36:** 453–60.

# Department of Internal Medicine
# Division of Respiratory Diseases

Kazuyoshi Kuwano, *Professor*
Akira Kojima, *Associate Professor*
Heiichi Yano, *Assistant Professor*
Yoshitsugu Nomoto, *Assistant Professor*
Hisakazu Tai, *Associate Professor*
Katsutoshi Nakayama, *Associate Professor*
Hiroshi Takeda, *Assistant Professor*
Jun Araya, *Assistant Professor*

## General Summary

We developed clinical and basic research concerning chronic obstructive pulmonary disease (COPD), bronchial asthma, pulmonary infection, pulmonary fibrosis, and lung cancer. Basic research should address clinical problems, and clinical research should establish novel treatments. We started clinical research concerning COPD 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. We specifically investigated the role of apoptosis, senescence, and autophagy in the pathogenesis of these devastating lung diseases.

## Research Activities

COPD: Clinical research concerning the incidence of COPD in patients with diabetes mellitus was developed, and data will soon be presented at a conference. Clinical research concerning the incidence of COPD in patients with cardiovascular diseases was started. The effect of steroid inhalation on oxidative stress in patients with COPD has been investigated.
Pulmonary infection: An investigation of biomarkers for infectious lung diseases was started. This study focused on the significance of procalcitonin in the diagnosis and treatment of pulmonary infection. The results of this study will be presented at the Japanese Respiratory Society Congress in 2010.
Lung cancer: Clinical research about the effect of nitroglycerin on chemotherapy in non-small cell lung cancer is in progress. 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.
Pulmonary fibrosis: Basic research is in progress concerning the mechanism of transforming growth factor-beta activation by integrin and its role in epithelial and mesenchymal cell interactions. We are also investigating the role of senescence and autophagy in pulmonary fibrosis and airway thickening.

### Publications

***Ouchi H, Fujita M, Ikegame S, Ye Q, Inoshima I, Harada E, Kuwano K, Nakanishi Y.*** The role of collagenases in experimental pulmonary fibrosis. *Pulm Pharmacol Ther* 2008; **21:** 401–8.

***Yamada M, Kuwano K, Maeyama T, Hamada N, Yoshimi M, Nakanishi Y, Kasper M.*** Dual-immunohistochemistry provides little evidence for epithelial-mesenchymal transition in pulmo-

nary fibrosis. *Histochem Cell Biol* 2008; **129:** 453–62.

***Hamada N, Maeyama T, Kawaguchi T, Yoshimi M, Fukumoto J, Yamada M, Yamada S, Kuwano K, Nakanishi Y.*** The role of high mobility group box1 in pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2008; **39:** 440–7.

***Yoshimi M, Maeyama T, Yamada M, Hamada N, Fukumoto J, Kawaguchi T, Kuwano K, Nakanishi Y.*** Recombinant human erythropoietin reduces epithelial cell apoptosis and attenuates bleomycin-induced pneumonitis in mice. *Respirology* 2008; **13:** 639–45.

***Tatsumi K, Shinozuka N, Nakayama K, Sekiya N, Kuriyama T, Fukuchi Y.*** Hichuekikito imporoves systemic inflammation and nutritional status in obstructive pulmonary disease. *J Am Geriatr Soc* 2009; **57:** 169–70.

***Nakao I, Kanaji S, Ohta S, Matsushita H, Arima K, Yuyama N, Yamaya M, Nakayama K, Kubo H, Watanabe M, Sagara H, Sugiyama K, Tanaka H, Toda S, Hayashi H, Inoue H, Hoshino T, Nakajima A, Inoue M, Suzuki K, Aizawa H, Okinami S, Nagai H, Hasegawa M, Fukuda T, Green ED, Izuhara K.*** Identification of pendrin as a common mediator for mucus production in bronchial asthma and chronic obstructive pulmonary disease. *J Immnol* 2008; **180:** 6262–9.

***Araya J, Kawabata Y, Jinho P, Uchiyama T, Ogata H, Sugita Y.*** Clinically occult subpleural fibrosis and acute interstitial pneumonia a precursor to idiopathic pulmonary fibrosis? *Respirology* 2008; **13:** 408–12.

***Akiba T, Marushima H, Takagi M, Odaka M, Harada J, Kobayashi S,Morikawa T.*** Preoperative evaluation of a tracheal bronchus by three-dimensional 64-row multidetector-row computed tomography (MDCT) bronchography and angiography: report of a case. *Surg Today* 2008; **38:** 841–3. Epub2008 Aug 28

***Shimizu K, Kikuchi K, Sasaki T, Takahashi N, Ohtsuka M, Ono Y, Hiramatsu K.*** Smqnr, a new chromosome-carried quinolone resistance gene in *Stenotrophomonas maltophilia. Antimicrob Agents and Chemother* 2008; **52:** 3823–5.

***Aizaki H, Morikawa K, Fukasawa M, Hara H, Inoue Y, Tani H, Saito K, Nishijima M, Hanada K, Matsuura Y, Lai MM, Miyamura T, Wakita T, Suzuki T.*** Critical role of virion-associated cholesterol and sphingolipid in hepatitis C virus infection. *J Virol* 2008; **82:** 5715–24.

# Department of Internal Medicine
# Division of General Medicine

Ken Hokkyo, *Professor*
Norio Tada, *Professor*
Hideaki Suzuki, *Associate Professor*
Masami Nemoto, *Associate Professor*
Futoshi Kotajima, *Assistant Professor*
Nobuyuki Furutani, *Assistant Professor*
Nobuakira Takeda, *Professor*
Akihiro Nishiyama, *Associate Professor*
Masato Matsushima, *Associate Professor*
Hiroshi Yoshida, *Associate Professor*
Chihiro Shikata, *Assistant Professor*
Jun Hiramoto, *Assistant Professor*

## Research Activities

*Division of General Medicine, The Jikei University Hospital*

Little is known about the relationship between anxiety and health-related quality of life (QOL) in the primary care setting. The objective of this study was to determine whether the degree of anxiety affects physical complaints and the QOL physical status scale. The State Trait Anxiety Inventory (STAI), the 36-item Short Form Health Survey (SF-36), and the Beck Depression Inventory II (BDI-II) were used to evaluate the degree of anxiety, health-related QOL scores, and degree of depressive symptoms, respectively. The subjects were 109 patients (64 men and 45 women) with a mean age of 41.8±13.8 years. Of the 25 physical complaints, general fatigue, vertigo, dizziness were correlated with anxiety. The physical component summary of the SF-36 has not shown a statistically significant relation with anxiety, after we adjusted for sex, age, and BDI-II score.

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

The effect of *shinrin-yoku* (forest-air bathing and walking) in patients with hypertension were investigated. Beneficial effects of the antiplatelet agent sarpogrelate were examined in an experimental model of heart failure due to myocardial infarction.

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

1. Study of factors of infection in elderly hospitalized patients

To investigate the relation between infection and several factors in elderly inpatients, we studied how infection is related to the nutritional state, administered drugs, and biochemical markers. We found that a poor nutritional state and the use of gastric acid—suppressing drugs promote infection in elderly inpatients.

2. Study of fever of unknown origin

We attempted to clarify the cause of fever of unknown origin by measuring white blood cells, the erythrocyte sedimentation rate, and levels of C-reactive protein, adenosine deaminase, 2–5 oligoadenylate synthetase, soluble interleukin-2 receptor, and procalcitonin. We found that viral infection can be distinguished from bacterial infection on the basis of the results of these measurements. Procalcitonin is useful for the diagnosis of Gram-negative rod sepsis.

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

1. Investigation of the role of general medicine on environmental health achievement
We established a new regional nutritional education system for patients with diabetes mellitus or metabolic syndrome in the Kashiwa area. This work received an award from the Kao Research Council for the Study of Healthcare Science in 2008. As a member of the local governance committee, we also participated in the development of local health care systems, *tokuteikenshin* and *tokuteihokenshidou*, in the Kashiwa area.

2. Studies of lipid metabolism and atherosclerosis

1) We started *in vitro* studies to clarify the mechanism by which diacylglycerol ingestion increases plasma serotonin and the relation of this phenomenon to the antiobesity effects of diacylglycerol, which was reported by us in the *Journal of Clinical Lipidology* last year.

2) An incubation study using bacteriophage was started with lipoprotein fractions to examine the antiviral effects of high-density lipoprotein.

3) Current issues of a low-density lipoprotein (LDL) homogenous assay were highlighted by lipoprotein analysis with our newly developed method of high-performance liquid chromatography.

4) The clinical significance of malondialdehyde-modified LDL, a clinical detector of oxidized LDL, was assessed.

5) The effects of statins on non—high-density lipoprotein cholesterol were examined in a multicenter randomized controlled trial, and these results were reported in *Atherosclerosis* in 2008.

### Publications

***Mamori S, Searashi Y, Matsushima M, Hashimoto K, Uetake S, Matsudaira H, Ito S, Nakajima H, Tajiri H.*** Serum type IV collagen level is predictive for esophageal varices in patients with severe alcoholic disease. *World J Gastroenterol* 2008; **14:** 2044–8.

***Yutaka O, Katsunori I, Ikuro A, Shinichi S, Kenichi D, Kumie I, Hidekatsu Y, Norio T.*** Characteristic comparison of triglyceride-rich remnant lipoprotein measurement between a new homogeneous assay (RemL-C) and a conventional immunoseparation method (RLP-C). *Lipids Health Dis* 2008; **17:** 18.

### Reviews and Books

***Yanai H, Tomono Y, Ito K, Furutani N, Yoshida H, Tada N.*** The underlying mechanisms for development of hypertension in the metabolic syndrome. *Nutr J* 2008; **17:** 10.

***Matsushima M.*** Introduction of the educational programs for the medical practitioners intended to clinical researches on the primary care settings; The Jikei University School of Medicine (in Japanese). *Rinsho Yakuri* 2008; **39:** 191–3.

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***Tada N.*** The diet for patients with dyslipidemia (in Japanese). *Lipid* 2008; **19:** 166–73.

***Tada N.*** What are the factors related to postprandial hyperlipidemia? (in Japanese). *Q and A de Wakaru Himan to Tonyobyo* 2008; **7:** 887–90.

***Tada N.*** The evidence of treatment for hypertriglyceridemia (in Japanese). *Gekkan Resident* 2009; **2:** 49–55.

# Department of Psychiatry

Kazuhiko Nakayama, *Professor*
Hiroshi Itoh, *Professor*
Hisatsugu Miyata, *Associate Professor*
Kazutaka Nukariya, *Assistant Professor*
Motohiro Ozone, *Assistant Professor*
Tatsuroh Nakanishi, *Assistant Professor*
Minako Koga, *Assistant Professor*
Hiroo Kasahara, *Professor*
Kei Nakamura, *Professor*
Hironari Sue, *Associate Professor*
Wataru Yamadera, *Assistant Professor*
Kazuya Ono, *Assistant Professor*
Toshihiko Hashizume, *Assistant Professor*

## General Summary

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

## Research Activities

*Psychopathology, psychotherapy and child study group*
The first study concerned the pathological structure of the mixed state of major depression and autistic disorders and that of self-injurious behavior from the viewpoint of nonadaptive defense mechanisms. The second study investigated background factors of a patient who has been absent from work because of mental disorders from the viewpoint of the mental health in the office. Third, we started a basic investigation of the psychological problems of patients with acquired immunodeficiency syndrome and began to develop diary training as a new treatment for Asperger disorders.

*Morita therapy group*
Guidelines for outpatient Morita therapy have been established. Studies have been continued on various topics, such as character profiles of patients with chronic depression, the relationship between panic disorder and generalized anxiety disorder from the aspect of patients' character and comorbidity, the subtypes of obsessive-compulsive disorder (OCD), and the "fall-in reaction" occurring in the process of mood or anxiety disorders. Also started this year were qualitative research on the life stories of patients with OCD and a study on factors in the recovery of patients with depression undergoing inpatient Morita therapy.

*Psychopharmacology group*
In basic research, the mechanism of the central actions of a new generation of psychotropic drugs was studied using microdialysis or radioimmunoassay, and the brain mechanisms of learning and memory underlying drug dependence were studied in rats. Clinical research involved studies of the clinical effectiveness and adverse events of new psychotropic drugs, positron emission computed tomography study of brain receptors in patients with mental disorders, molecular biological studies of mental disorders in corroboration with the department of virology, and genetic research on antipsychotic

agent—induced akathisia.

*Psychophysiology group*
Studies examined the effects of *yokukansan* and quetiapine on the cyclic alternating pattern as an index of sleep structures, the effects of nasal continuous positive airway pressure in patients with obstructive sleep apnea syndrome, the effects of cognitive behavioral therapy and Morita therapy on outpatients with psychophysiological insomnia, and the clinical effects of modafinil on central hypersomnia. Furthermore, a clinical investigation examined sleep disturbance in patients with functional gastrointestinal disorders.

*Psychogeriatric group*
First, epidemiological studies were performed in Itoigawa City, Niigata Prefecture, focusing on the current use and costs of nursing care insurance and on predictors of mortality risk in the elderly. Second, studies of mental disorders in patients with breast cancer were performed in collaboration with the general hospital psychiatry research group and the department of surgery. In addition, we started a clinic specializing in dementia.

*General hospital psychiatry*
We have continued therapy based on cognitive-behavioral therapy aimed at preventing recurrences of depression. To improve this therapy, we introduced a computer system for more effective presentations and introduced more precise sleep-evaluation methods to supplement the existing evaluation system. Furthermore, we investigated new indications for this treatment, such as atypical depression and insomnia, and the supportive mental care for cancer patients, their family, and medical staff.

*Clinical electroencephalography group*
Studies were performed on the clinical significance of the 6-Hz spike and wave on electroencephalography, the characteristics of "social withdrawal" caused by epilepsy, the subjective and objective premonitory symptoms as epileptic prodromes, and medications for patients with epilepsy and psychotic symptoms. Furthermore, we reported on a case of sleep-related abnormal behavior resembling epilepsy.

*Clinical psychology group*
Studies examined psychotherapeutic processes, the treatment techniques of cognitive behavior therapy and Morita therapy, and the characteristics of developmental disorders and higher brain dysfunctions using psychological assessments. Furthermore, we educated graduate students and medical residents.

**Publications**

***Nakayama K, Katsu H, Kitasumi K.*** Effect of distigmine bromude on the centralcholinergic system. *J Psychopharmacol* 2008; **10:** 1-4.
***Miyata H, Hironaka N (ERATO), Takada K (Teikyo***

***Univ), Miyasato K (Funomori Clin), Nakamura K (Int Univ), Yanagita T.*** Psychosocial withdrawal characteristics of nicotine compared with alcohol and caffeine. *Ann NY Acad Sci* 2008; **1139:** 458–65.

***Ozone M, Yagi A, Itoh H, Tamura Y, Inoue Y, Uchimura N, Sasaki M, Shimizu T, Terzano MG, Parrino L.*** Effects of zolpidem on cyclic alternating pattern, an objective marker of sleep instability, in Japanese patients with psychophysiological insomnia: a randomized crossover comparative study with placebo. *Pharmacopsychiatry* 2008; **41**: 106–14.

***Shinagawa S, Toyota Y, Ishikawa T, Fukuhara R, Hokoishi K, Komori K, Tanimukai S, Ikeda M.*** ACCCognitive function and psychiatric symptoms in early-onset frontotemporal dementia and late-onset frontotemporal dementia. *Dement Geriatr Cogn Disord* 2008; **25:** 439–44.

***Nakayama K.*** Female's anxiety and Moritatherapy (in Japanese). *Josei Shinshin Igakukaishi* 2009; **14:** 27–30.

***Miyata H.*** Psychopharmacological characteristics of second generation anti-psychotic drugs: beneficial effects on reward and motivation (in Japanese). *Brain* 2008; **12:** 1–6.

***Yamadera W, Itoh H, Inoue Y, Kanbayashi S, Tagaya H, Kamei Y.*** Guidline of diagnosis and treatment of insomnia (in Japanese). *Suimin Iryo* 2008; **2:** 285–9.

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***Oh T, Shinagawa S, Nakamura S, Toh K, Shigeta M.*** Conparison study on BPSD of patients with dementia between Japan and China (in Japanese). *Nihon Hokenkagaku Gakkaishi* 2008; **10:** 12–9.

***Ochiai Y, Nakayama K.*** Prominent effects of sulpride on adjustment disorder after surgical operation for bladder cancer. *Seishinka* 2008; **12:** 324–7.

## Reviews and Books

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***Nakayama K.*** Reconsideration of treatment of depression (in Japanese). *Kokorono Kurinikku* 2008; **9:** 28–35.

***Miyata H.*** The role of smoking and nicotine in neuropsychiatric disorders: evidence for therapeutic role of nicotinic acetylcholine receptors (in Japanese). *Jpn J Nuropsychopharmacol* 2008; **28:** 149–58.

***Miyata H.*** Pharmacology of second generation antipsychotics: consideration of clinical significance of D2 receptor occupancy (in Japanese). *Igakunoayumi* 2008; **227:** 497–501.

***Yamadera W.*** Sleep disorders (in Japanese). *Saishinigaku* 2008; **56:** 69–81.

***Ozone M, Itoh H.*** Sleep deprivation in jet lag syndrome (in Japanese). *Rinsho Noha* 2008; **50:** 710–17.

***Nakamura K.*** Personality disorder in workplace (in Japanese). *Nihon Ijishinpo* 2008; **4374:** 42–5.

***Shinagawa S, Shigeta M.*** Prevention of dementia by medication (in Japanese). *Modern Physican* 2008; **27:** 1485–9.

***Nakamura S.*** Prevention study on dementia in Itoigawa city of Nigata prefecture (in Japanese). *Modern Physican* 2008; **28:** 1507–1510.

***Ochiai Y, Kasahara H.*** Early intervention in Alzheimer disease: characteristic symptoms and course (in Japanese). *Schizophreania Frontia* 2009; **10:** 29–32.

# Department of Pediatrics

Hiroyuki Ida, *Professor*
Fumiyuki Ito, *Professor*
Makoto Nakazawa, *Professor*
Tohya Ohashi, *Professor*
Makiko Okuyama, *Associate Professor*
Hisashi Tamaki, *Associate Professor*
Toshio Katsunuma, *Associate Professor*
Ichiro Miyata, *Associate Professor*
Masako Fujiwara, *Assistant Professor*
Yoshihiro Saito, *Assistant Professor*
Masaki Shimizu, *Assistant Professor*
Hiroshi Kobayashi, *Assistant Professor*
Yuki Yuza, *Assistant Professor*
Masahisa Kobayashi, *Assistant Professor*
Masakatsu Kubo, *Professor*
Nobuo Usui, *Professor*
Yasutaka Hoshi, *Professor*
Takashi Kaneko, *Associate Professor*
Kiyoshi Ogawa, *Associate Professor*
Shin-ichiro Hamano, *Associate Professor*
Yasuyuki Wada, *Associate Professor*
Mitsuyoshi Urashima, *Associate Professor*
Yoko Kato, *Assistant Professor*
Akinori Shukuya, *Assistant Professor*
Hiroshi Tachimoto, *Assistant Professor*
Masaharu Akiyama, *Assistant Professor*
Yuichi Fuyama, *Assistant Professor*

## General Summary

We have 9 subspeciality research groups: (1) the Medical Genetics, Congenital Metabolic Diseases, Endocrinology, Gastroenterology and Hepatology group; (2) the Allergy and Immunology group; (3) the Neurology group; (4) the Hematology and Oncology group; (5) the Cardiology group; (6) the Infectious Diseases group; (7) the Neonatology group; (8) the Nephrology group; and (9) the Pediatric Psychiatry group. The ultimate goal of these subspeciality groups is to supply practical benefits to patients and their families through basic and clinical research. To accomplish this goal, cooperation and a high degree of motivation for research are need.

## Research Activities

*Medical Genetics, Congenital Metabolic Diseases, Endocrinology, Gastroenterology, and Hepatology group*

We focused on research concerning medical genetics, congenital metabolic diseases, endocrinology, gastroenterology, and hepatology. Accomplishments of our group this year are as follows.

1. In enzyme replacement therapy for lysosomal storage diseases, we developed a novel immune tolerance induction method for enzymes.
2. We developed a novel gene therapy for lysosomal storage diseases using a murine model.
3. In the field of endocrinology, we surgically prepared rats with left ventricular heart failure by banding the aorta, and analyzed the expression patterns of urocortin 2, urocortin 3, and corticotropin releasing factor receptor $2\alpha$ in their brains.
4. We evaluated the usefulness of a new diagnostic kit for norovirus infection and complications of norovirus infection. We also updated safety information regarding tumor necrosis factor blockers for Crohn disease.
5. We reported on a fetus having the sirenomelia sequence with a reciprocal *de novo*

translocation. We refined the breakpoints of each derivative chromosome and analyzed the etiological mechanisms.
6. We are attempting to develop a murine model of malaria infection.

*Neurology group*
We focused on research activities concerning higher cortical dysfunction in acquired brain injury (ABI) rehabilitation during childhood. Higher cortical dysfunction is an important aspect in pediatric ABI rehabilitation. Recently, higher cortical dysfunction has attracted attention, and many trials have been performed, but most trials have involved only adults. Higher cortical dysfunction seems to show better recovery in children than in adults because of the plasticity of a child's brain. The main causes of ABI in children are traumatic brain injury (TBI) and acute encephalitis/encephalopathy. The characteristic symptoms are memory disturbance and attention deficit in TBI, and vision problems in acute encephalitis/encephalopathy. It is important for children who have higher cortical dysfunction to be educated with special programs and to be cared for with cooperation among rehabilitation centers, schools, and their families.

*Cardiology group*
For basic research, a mouse model of right heart failure was created to investigate gene expression and physiological changes in right ventricular remodeling. Because many questions remain regarding the effect of right heart failure upon various organs, we have undertaken joint studies with the Cardiology group and the Pediatric Endocrinology group. We have also studied the growth of the pulmonary artery using a model mouse of pulmonary artery stenosis created by pulmonary artery banding. Our clinical research has examined: 1) magnesium kinetics in pediatric cardiology, 2) the treatment of pediatric arrhythmia using magnesium, 3) the secretion and kinetics of atrial natriuretic peptide and brain natriuretic peptide in pediatric cardiac diseases, 4) cardiac lesions of Fabry disease, 5) hemodynamics after the Fontan operation, and 6) postoperative antithrombotic therapy for congenital heart disease.

*Infectious disease group*
In an effort to respond to advances in the field of infection and immunology, the Research Group of Infection and Immunity carefully evaluates clinical cases from affiliated hospitals to incorporate present needs in clinical practice into individual research projects. Our research includes 3 main fields: 1) pediatric rheumatoid disease, 2) immunology and immunodeficiency, and 3) bacterial and viral infectious diseases. In recent years, diagnostics and therapeutics in pediatric rheumatology have improved significantly. More-detailed follow-up has become possible by combining various markers of disease activity, allowing normal growth and development to be maintained while disease activity remains well controlled.
For research on immunodeficiency, we aim to develop the most advanced forms of therapy, such as gene therapy, using our extensive clinical experience, including many cases of bone marrow transplantation.
For research on infectious diseases, we have substantial data on bacterial meningitis,

sepsis, and other diseases collected in this department for use in clinical research. Recently, we have focused on the species-specific diversity of bacterial 16S ribosomal RNA sequences. We believe that examining the sequences of bacterial RNA in the blood of patients with diseases of unknown etiology will pave the way to the identification of bacterial species and assist in the investigation of the causes of disease. We are also evaluating the in vivo kinetics of antibiotics specific for children. Each member of our group has their particular areas of expertise and continuously aims to gain a wider range of knowledge in the field of infection and immunology.

*Nephrology group*

We have focused on research concerning intractable nephrotic syndrome, urinary tract infection, and acute dialysis. In urinary tract infection, we analyzed the clinical manifestations and frequency of breakthrough infection in patients with high-grade vesicouteric reflux (grade≥III). We demonstrated the efficacy of prophylaxis with cefaclor.

**Publications**


***Tajima A, Yokoi T, Ariga M, Ito T, Kaneshiro E, Eto Y, Ida H.*** Clinical and genetic study of Japanese patients with type 3 Gaucher disease. *Mol Genet Metab* 2009; **97:** 272–7.

***Ohashi T, Iizuka S, Ida H, Eto Y.*** Reduced alpha-Gal A enzyme activity in Fabry fibroblast cells and Fabry mice tissues induced by serum from antibody positive patients with Fabry disease. *Mol Genet Metab* 2008; **94:** 313–8.

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***Imada Y, Fujimoto M, Hirata K, Hirota T, Suzuki Y, Saito H, Matsumoto K, Akazawa A, Katsunuma T, Yoshihara S, Ebisawa M, Shibasaki M, Arinami T, Tamari M, Noguchi E.*** Large scale genotyping study for asthma in the Japanese population. *BMC Res Notes* 2009; **2:** 54.

***Kondo-Endo K, Ohashi Y, Nakagawa H, Katsunuma T, Ohya Y, Kamibeppu K, Masuko I.*** Development and validation of a questionnaire measuring quality of life in primary caregivers of children with atopic dermatitis (QPCAD). *Br J Dermatol* 2009; **Apr 29.**

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***Kobayashi S, Murayama S, Takanashi S, Takahashi K, Miyatsuka S, Fujita T, Ichinohe S, Koike Y, Kohagizawa T, Mori H, Deguchi Y, Higuchi K, Wakasugi H, Sato T, Wada Y, Nagata M, Okabe N, Tatsuzawa O.*** Clinical features and prognoses of 23 patients with chronic granulomatous disease followed for 21 years by a single hospital in Japan. *Eur J Pediatr* 2008; **167:** 1389–94.

***Akiyama M, Yuza Y, Yokokawa Y, Yokoi K, Ariga M, Eto Y.*** Differences in CTG triplet repeat expansion in leukemic cells and normal lymphocytes from a 14-year-old female with congenital myotonic dystrophy. *Pediatr Blood Cancer* 2008; **51:** 563–5.

***Yamada O, Kawauchi K, Akiyama M, Ozaki K, Motoji T, Adachi, Aiwaka.*** Leukemic cells with increased telomerase activity exhibit resistance to imatinib. *Leuk Lymph* 2008; **49:** 1168–77.

***Ozaki K, Nakatake M, Akiyama M, Kawauchi K,***

***Matsuoka R.*** Multistep regulation of telomerase during differentiation of HL60 cells. *J Leuk Biol* 2008; **83:** 1240–48.

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***kiyama, M, Yamada O, Agawa M, Yuza Y, Yanagisawa T, Eto Y, Yamada H.*** Effects of prednisolone on specifically expressed genes in pediatric acute B-lymphoblastic leukemia. *J Pediatr Hematol Oncol* 2008; **30:** 313–6.

## Reviews and Books

***Saito H.*** Progress in allergy signal research on mast cells: systemic approach to mast cell biology in allergic diseases. *J Pharmacol Sci* 2008; **106:** 341–6.

# Department of Dermatology

Hidemi Nakagawa, *Professor*
Mariko Honda, *Professor*
Arihito Ota, *Assistant Professor*
Masaaki Kawase, *Assistant Professor*
Ryoichi Kamide, *Professor*
Takaoki Ishiji, *Associate Professor*
Tsunemichi Takeuchi, *Assistant Professor*
Matsuo Koma, *Assistant Professor*

## General Summary

We have organized special clinics for selected skin diseases, including viral diseases, neurofibromatosis (NF) type 1, atopic dermatitis, psoriasis, collagen vascular diseases 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 cyclosorin, methotrexate, and etretinate, in addition to topical vitamin D3 and steroids have been used depending on disease severity and the degree of quality of life (QOL) impairment in individual patients. In addition, phototherapy, including psoralen ultraviolet (UV) A, narrow-band UVB, and the 308-nm excimer lamp, are known to be effective and are administered in our newly organized skin-care clinic. We have evaluated patients' QOL reflecting social background and elaborated the Japanese version of the Psoriasis Disability Index. In a special psoriasis clinic, we select patient-based treatments to satisfy patients' demands. Clinical trials of new biologic agents, including infliximab, adalimumab, and ustekinumab, have been performed. We have organized meetings twice a year in the auditorium of our university with patients who have psoriasis in the Tokyo area to enhance the patients' knowledge about psoriasis.

*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, it has been suggested that psychosocial factors can exacerbate atopic dermatitis. Therefore, we are trying to treat patients on the basis of QOL issues as well as on the basis of evidence-based medicine. We try to obtain accurate medical histories and information about QOL impairment from each patient. To support such an approach, we have organized skin-care lessons at the skin care clinic twice weekly and the atopic dermatitis forum, which includes lectures and group meetings monthly. For basic clinical research, the levels of substance P and interleukin 31 related to pruritus in atopic dermatitis are being evaluated according to disease severity. Clinical trials of a topical nuclear factor $\kappa$B decoy and a $\kappa$-opioid agonist have been performed.

*Malignant skin tumors*

We have been studying clinical courses and postoperative outcomes of 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 according to established therapeutic guidelines. For the accurate diagnosis of pigmented tumors, we always perform dermoscopic examinations. Sentinel lymph-node biopsy is performed, especially for patients with stage II and III melanoma. We are participating in cooperative clinical research on maintenance therapy with local injections of interferon $\beta$.

*NF1*

Because the registered number of patients in our NF1 clinic is the largest in Japan and because many patients from all over Japan are referred to us, we concentrate on the accurate diagnosis of NF1, the improvement of impaired QOL by resection of neurofibromas, and long-term follow-up. The lifetime risk of malignant peripheral nerve sheath tumor (MPNST) associated with NF 1 is estimated to be 10%, and surgical removal is the most effective treatment; for these reasons, MPNST should be detected as early as possible.

Recently, diffusion-weighed imaging (DWI) techniques have been used to improve the accuracy of diagnosis and have become standard procedures for detecting malignant tumors of the breast and prostate and tumors metastatic to the bone and liver. We evaluated DWI findings and examined their correlation with pathological findings for 10 patients with deeply situated hard tumors. Six tumors, which showed high signal intensity (even in part of the tumor) on high-b DWI, were shown by pathological examination to be MPNSTs. On the other hand, 4 tumors with low signal intensity or lower signals on high-b DWI than on low-b DWI were shown to be neurofibromas. We have demonstrated that DWI is more useful for detecting MPNSTs than is conventional MRI because of its high sensitivity and specificity.

*Herpes virus infection*

1. Herpes simplex virus

We treat patients with genital herpes and refractory oral herpes. Rapid diagnostic procedures with immunohistochemical staining and monoclonal antibodies against herpes simplex virus (HSV)-1, HSV-2, and varicella-zoster virus (VZV) are performed in this clinic. After the diagnosis is confirmed, suppressive therapy with valaciclovir is started to improve the impaired QOL. We have confirmed that the loop-mediated isothermal amplification method is an excellent alternative to conventional polymerase chain reaction assays for the rapid detection of HSV-1 and 2 and VZV in clinical specimens.

A survey of QOL in patients with recurrent genital herpes and drug sensitivities derived from HSV from recurrent genital herpes are now in progress.

2. Herpes zoster and postherpetic neuralgia

The initial treatment for herpes zoster (HZ) and postherpetic neuralgia (PHN) is performed in this clinic. Famciclovir is the oral prodrug of penciclovir, an agent that

has demonstrated antiviral activity against HSV-1 and 2 and VZV. It is as effective as acyclovir or valaciclovir for the treatment of HZ and was eventually approved for use in Japan. We are now evaluating the efficacy and safety of this drug. PHN is a major sequela of VZV infection and impairs the patients' QOL. To control PHN, we are prescribing selective serotonin reuptake inhibitors and investigating the efficacy of other new drugs.

*Human papillomavirus infection*

In addition to ordinary cryotherapy, topical vitamin D3 and salicilic acid have been used to treat viral warts. In addition, contact immnunotherapy with squaric acid dibutyl ester and $CO_2$ laser evaporation have been also applied to refractory viral warts. Typing of HPV with the polymerase chain reaction method has been performed regularly for condylomas and rare viral warts. Imiquimod cream (5%) is now available for the treatment of condyloma accuminatum.

*Collagen vascular diseases*

Detailed, periodic follow-up is performed for patients with systemic lupus erythematosus, systemic sclerosis, dermatomyositis, localized scleroderma, Beċhet disease, autoimmune vascular diseases.

*Contact dermatitis/drug eruption*

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

*Laser*

This year, about 100 patients per month were treated with lasers in the Dermatology Laser Unit. The Q-switched ruby laser is useful for treating nevus Ota because of its selective photothermolysis. Superficial pigmented lesions, such as senile pigment freckles, are usually successfully treated in one session. Nevus spilus is difficult to treat with the Q-switched ruby laser because it often recurs 1 to 2 months after treatment. The efficacy of a pulsed dye laser for treating hemangiomas and teleangiectasia depends on the 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 intensity of the strawberry mark can be reduced if treatment is started before the age of 6 months. The recently introduced V-beam is expected to be effective for refractory vascular lesions. Because the ultrapulse $CO_2$ laser has higher energy and a shorter pulse width, it can vaporize the skin at a fixed depth and can be used to quickly remove actinic keratosis, seborrhoeic keratosis, syringoma, and epidermal nevus.

*Skin care clinic*

Narrow-band UVB irradiation is performed for patients with psoriasis, atopic dermatitis, prurigo nodularis, vitiligo, and cutaneous T-cell lymphomas. A 308-nm excimer lamp is also used. Patients may also attend special clinics and learn about skin care, therapy make-up, acne care, mental care, and *kampo* medicine.

*Self-assessment*

Psoriasis: We have selected therapies on the basis of their risk/benefit ratio to improve patients' QOL and treatment compliance. Phototherapy with narrow-band UVB and the 308-nm excimer lamp have been introduced. Clinical trials of new biologic agents have been performed.

NF: Many patients with NF I are still being referred to our special clinic. We are now doing inheritance consultation for pediatric patients. Different types of neurofibromas are surgically removed at the inpatient and outpatient clinics to enhance QOL.

Herpes virus infection: We have developed the loop-mediated isothermal amplification method for accurate and rapid diagnosis. Selective serotonin reuptake inhibitors have proven effective for the treatment of PHN.

HPV infections: We have used new treatments, including topical vitamin D3, in addition to ordinary surgical treatments, for viral warts. Typing of HPV is also regularly performed.

Contact dermatitis: Tests are regularly performed for causal chemicals, environmental allergens, drugs, and foods in patients with contact dermatitis and drug eruption.

Atopic dermatitis: We have been trying to treat patients according to the established guidelines and the degree of QOL impairment. The psychosocial background of patients is also considered. To help patients' understanding, each month we have been organizing atopic dermatitis forums that include lectures and group meetings. Basic research has focused on pruritogens, such as substance P and IL-31.

Malignant skin tumors: We have been treating many patients with skin cancers, including melanomas and extramammary Paget disease, by surgical operation combined with sentinel lymph-node biopsies and chemotherapy.

Laser: We have been treating many patients using several different types of laser.

Collagen vascular diseases: Detailed, 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.

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# Department of Radiology

Kunihiko Fukuda, *Professor*
Junta Harada, *Professor*
Tetsuhisa Yamada, *Associate Professor*
Yukio Miyamoto, *Associate Professor*
Hiroya Ojiri, *Associate Professor*
Manabu Aoki, *Assistant Professor*
Chihiro Kanehira, *Professor*
Toru Sekiya, *Associate Professor*
Shunichi Sadaoka, *Associate Professor*
Mayuki Uchiyama, *Associate Professor*
Takeo Irie, *Assistant Professor*
Norio Nakata, *Assistant Professor*

## Research Activities

*The division of diagnostic imaging*

1. Multidetector-row computed tomography as a preoperative evaluation for partial hepatectomy

Liver parenchyma/volumetry, vascular structures, and the biliary system should be assessed before partial hepatectomy is performed. To establish an imaging strategy before partial hepatectomy, we obtain imaging data of the liver in both the arterial and portal phases with dynamic computed tomography (CT) after drip infusion cholangiography-CT. Both 2-dimensional (D) and 3D displays of the biliary system and vascular structures are provided to surgeons.

2. Evaluation of the lymphatic system of the trunk on heavily T2-weighted images

A study was performed to evaluate the usefulness of nonenhanced 3D heavily T2-weighted images obtained with 2D prospective acquisition and correction in the visualization of the lymphatic system of the trunk.

3. The timing of liver tumor imaging with Sonazoid

In a collaborative study with the division of gastroenterology and hepatology, department of internal medicine, we investigated the timing of liver tumor imaging with Sonazoid (Daiichi Sankyo, Tokyo), which is a second-generation sonographic contrast material. With a low mechanical index (MI) the ability of Sonazoid to depict the internal structure of blood vessels immediately after arterial injection was inferior to that of Levovist (Schering AG, Berlin, Germany), a first-generation conventional contrast medium, but with a high MI the sensitivity of Sonazoid was similar to that of Levovist. On the other hand, in focal nodular hyperplasia and adenoma, tumors were enhanced to a similar degree as was the neighboring liver parenchyma, whereas in hepatocellular carcinoma and angioma, tumors were depicted as contrasting deficits with Kupffer imaging 10 minutes after the injection of contrast media in the low MI reduction. These findings suggest that Kupffer imaging can be performed with an ultrasonographic contrast medium.

4. In collaboration with the division of rheumatology, department of internal medicine, we found that a change in the vascularity of the joint, as observed with color Doppler imaging, correlated with the condition of patients with rheumatoid arthritis and indicated the effect of treatment.

5. B flow ultrasonography for the diagnosis of renal artery stenosis: Clinical significance and comparison with other modalities

6. Fatty acid metabolism in the hearts of rats with renal failure

Cardiovascular disease is the best predictor of mortality in patients with chronic renal disease. The imaging of fatty acid analog is useful for diagnosing alterations of myocardial metabolism due to uremia and dialysis. We investigated the metabolism of iodine-125—labeled beta-methyl iodophenyl pentadecanoic acid (BMIPP) in the hearts of rats with renal failure. We compared the accumulation of I-125 BMIPP in the rat heart and pathological findings observed with transmission electron microscopy.

7. Multicenter trial confirmed the effectiveness of strontium-89 for palliative treatment in patients with multiple bone metastases

The bone-seeking radiopharmaceutical Sr-89 has been used in the palliative treatment of patients with bone pain caused by bone metastases. Sr-89 is a suitable isotope because it is a pure beta emitter. We obtained Sr-89 images with bremsstrahlung in patients 1 week after injection. The imaging of Sr-89 had not been previously reported. We participated in a multicenter trial to confirm the effectiveness of Sr-89 in combination with zoledronic acid and other anticancer agents.

8. Investigation of the physical properties of microcatheters smaller than 2.2 Fr

Various types of medical equipment are used for interventional radiology, and microcatheters are required to reach narrow, distal vessels for such techniques as transcatheter arterial embolization. We have previously reported the physical properties of an advanced microcatheter. The physical properties of microcatheters with tip diameters of 1.8 to 2.2 Fr were reviewed. We measured tip hardness, the smoothness of the interior and exterior surfaces, the flow rate, flexibility of the guide wire, the ability to maintain shape, resistance to kinking, visibility, intensity of pulling, and pressure resistance. The apical flexibility of the catheters was good, but flow rate, visibility, and pressure resistance were problematic.

*The division of radiation therapy*

1. The efficacy of re-irradiation after systemic treatment for recurrent disease

For patients with recurrence or metastatic lesions after radiotherapy, the efficacy of re-irradiation over the tolerance dose of normal tissues was examined. In 50% of patients, re-irradiation was successful for palliative therapy and maintained quality of life.

2. Relationship of bronchiolitis obliterans with organizing pneumonia syndrome and hormonal therapy after breast-conserving therapy

We examined the relationship of bronchiolitis obliterans with organizing pneumonia syndrome and factors in complications during systemic therapy after breast-conserving therapy. The incidence of bronchiolitis obliterans with organizing pneumonia syndrome in our hospital was 2.3%. Our findings suggest that patient age and the starting time of hormonal therapy are correlated with the risk of complications.

3. Randomized controlled trial of a trimodality protocol for high-risk prostate cancer

Many investigators have recently used a trimodality protocol (high dose rate prostate brachytherapy, external beam radiotherapy, and hormonal therapy) to treat high-risk prostate cancer. The optimal duration of hormonal therapy combined with radiotherapy has remained controversial. We are planning a randomized trial to determine the

optimal duration of hormonal therapy in a trimodality protocol for high-risk prostate cancer. The duration of neoadjuvant hormonal therapy is 6 months. Two arms will include adjuvant hormonal therapy for 6 months or for 2 years. We plan to start this randomized controlled trial in 2010.

4. Radiation therapy for primary orbital mucosa-associated lymphoid tissue lymphoma

From 2000 through 2007, 13 patients (8 men and 5 women; age range, 27 to 77 years; median age, 53 years) with orbital mucosa-associated lymphoid tissue lymphoma were treated with radiotherapy alone (total dose, 30 to 38 Gy; median dose, 31.6 Gy). Complete remission was obtained in 11 patients, and partial remission was achieved in 2 patients. Local control was achieved in all patients. Cataract surgery was required for 4 patients who received radiation without lens block. Radiotherapy was a safe and effective treatment.

### Publications

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# Department of Surgery
# Division of Digestive Surgery

Katsuhiko Yanaga, *Professor*
Kazuhiko Yoshida, *Professor*
Nobuyoshi Hanyu, *Associate Professor*
Norio Mitsumori, *Associate Professor*
Kazuo Matai, *Associate Professor*
Yoshiyuki Furukawa, *Assistant Professor*
Kouji Nakada, *Assistant Professor*
Yuichi Ishida, *Assistant Professor*
Takeyuki Misawa, *Assistant Professor*
Yoshiaki Kita, *Assistant Professor*
Hidejirou Kawahara, *Assistant Professor*
Naoto Takahashi, *Assistant Professor*
Hideyuki Kashiwagi, *Professor*
Susumu Kobayashi, *Professor*
Tetsuji Fujita, *Associate Professor*
Tomoyoshi Okamoto, *Associate Professor*
Akira Yanagisawa, *Assistant Professor*
Yuji Ishii, *Assistant Professor*
Shuzo Kono, *Assistant Professor*
Yoichi Toyama, *Assistant Professor*
Yoshio Ishibashi, *Assistant Professor*
Noburo Omura, *Assistant Professor*
Yoshiyuki Hoya, *Assistant Professor*

## General Summary

Last year, an interesting paper appeared in *The Lancet* which reported that 234 million major surgical procedures are performed worldwide each year, or 1 for every 25 people. This figure is more than twice that of yearly births and 7 times the 33.2 million people infected with human immunodeficiency virus. The increasing number of operations is associated with the introduction of new surgical techniques and refinements in perioperative care, such as laparoscopic cholecystectomy and a less-liberal fluid regimen for fast-track colonic surgery. These advances could be achieved by performing high-quality studies, including randomized controlled trials. Important scientific findings derived from experimental and clinical research should be published in academic journals.

To assess trends in our surgical research activity, we performed an advanced Web search with Medline using several key words, such as "Jikei," "surgery," and "English language." The search yielded 59 peer-reviewed articles from April 1, 2008, to March 31, 2009, a higher number than in any previous year. For example, the number of articles published from April 1, 2004 to March 31, 2005, was only 22. However, when the search is limited to core clinical journals, such as *Annals of Surgery* and *Archives of Surgery,* only 24 articles are found over a 10–year period. Consequently, we should continue to try to increase the quality of our research.

## Research Activities

*Upper gastrointestinal surgery*

The advantages and disadvantages of each method of laparoscopic surgery for achalasia and reflux esophagitis were carefully assessed, because the reputation for our technique has enabled us to evaluate many patients in spite of the rare nature of these diseases. Basic research in esophageal cancer has led us to discover molecular markers indicating prognosis.

We have established a new technique of sentinel node navigation surgery without radioisotopes for early gastric cancer using indocyanine green under infrared ray observation. A multicenter trial to evaluate our technique with infrared ray laparoscopy systems has been performed. The sentinel node detection rate, accuracy, and sensitivity were 100%. This method is safe and efficient.

*Colorectal surgery*

To improve the quality of laparoscopic operations we are evaluating the usefulness and reliability of the Virtual Reality Surgical Simulator for laparoscopic colectomy. We are also examining the relationship between the reactions of various immunoglobulins in the serum of patients with cancer by means of enzyme-linked immunosorbent assay and several factors relevant to cancer status. We are using the [$^{13}$C]-breath test to evaluate bowel function after colorectal surgery and to determine the appropriate duration of postoperative bowel rest. Preoperative diagnosis of lymph-node metastasis for colorectal cancer with diffusion magnetic resonance imaging (D-MRI) is ongoing. A total of 119 patients (52 with rectal cancer and 67 with colon cancer) were enrolled. Lymph-node metastases were judged with D-MRI and were compared with the pathological results. The form of metastasis was classified as abundant or scarce. We had discussed the results at the end of 1 year (period I, n=79) and re-audited the sensitivity and specificity after our meeting (period II, n=40). It was related with the ability of D-MRI to detect metastasis (period I: sensitivity=61%, specificity=73%, positive predictive value [PPV]=55%, and negative predictive value [NPV]=77%; period II: sensitivity=79%, specificity=95%, PPV=94%, and NPV=83%). The specificity and PPV for period II were significantly higher than those for period I ($p<0.05$). In period I and period II, respectively, the mean diameters of lymph nodes that D-MRI indicated were metastatic (i.e., "positive") were 10.3±5.4 mm (range, 3–28 mm; 32 nodes) and 9.1±3.0 mm (range, 4–14 mm; 16 nodes); those of true-positive nodes were 11.5±6.2 mm (range, 4–28 mm; 18 nodes) and 9.2±3.1 mm (range, 4–14 mm; 15 nodes); and those of false-positive nodes were 6±3.8 mm (range, 3–14 mm; 14 nodes) and 8 mm (1 node). On the other hand, the diameters of lymph nodes that D-MRI indicated were nonmetastatic (i.e., "negative") in period I and period II, respectively, were 5.9±2.4 mm (range, 3–16 mm; 47 nodes) and 5.7±2.8 mm (range, 2–15 mm; 24 nodes); those of true-negative nodes were 5.9±2.1 mm (range, 3–16 mm; 36 nodes) and 5.3±2.1 mm (range, 2–8 mm; 20 nodes); and those of false-negative nodes were 5.7±2.7 mm (range, 3–12 mm; 11 nodes) and 7.8±4.9 mm (range, 4–15 mm; 4 nodes). We have concluded that hot nodules with a diameter 9 mm or greater are clearly metastatic.

*Hepatobiliary and pancreatic surgery*

The outlines of our main research activities in the field of hepatobiliary and pancreatic surgery are as follows: 1) living donor liver transplantation (LDLT), regenerative medicine, and artificial liver (especially, implantable artificial liver); 2) chemotherapy for advanced pancreatic cancer; 3) expansion of surgical indications for multiple hepatic tumors by hepatic resection; and 4) laparoscopic resection of the liver, pancreas, and spleen.

The first LDLT was successfully performed for a patient with cirrhosis and postnecrotic hepatocellular carcinoma on February 9, 2007. Our sixth LDLT was performed for a patient with primary biliary cirrhosis on January 16, 2009. All six recipients were discharged 19 to 32 days after surgery and had a good clinical course. Our ongoing research on regenerative medicine and artificial organs is expected to have a synergistic effect on liver transplantation medicine. We have performed translational research through combination chemotherapy with gemcitabine and the naive protease inhibitor FUT-175, which has the dual functions of nuclear factor $\kappa$B inhibition and apoptosis induction in pancreatic cancer cell lines. Regarding other issues described above, clinical and experimental studies are ongoing after being approved by the Ethics Committee of The Jikei University.

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

Toshiaki Morikawa, *Professor*
Tadashi Akiba, *Associate Professor*
Kozo Nakanishi, *Assistant Professor*
Shuji Sato, *Assistant Professor*
Ken Uchida, *Professor*
Hisaki Fukushima, *Assistant Professor*
Toru Kuroda, *Assistant Professor*
Hiroshi Takeyama, *Assistant Professor*
Satoki Kinoshita, *Assistant Professor*
Yasuo Toriumi, *Assistant Professor*

## General Summary

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

## Research Activities

*Chest surgery*

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

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

Operative procedures, including wedge resection, segmentectomy, lobectomy, and pneumonectomy of the lung, are all safely performed, as are resection of mediastinal tumors or the thymus. Surgery for lung cancer requires much more advanced skills and oncological considerations, which have also been independently developed. Of the mediastinal procedures, thymectomy is usually performed via thoracoscopy rather than via a conventional median sternotomy. In our department more than 90% of the chest operations are performed via thoracoscopy, 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 video-assisted lung cancer surgery with open surgery, an evaluation of video-assisted surgery for bullous lung diseases in elderly persons 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 thoracoscopic diagnosis of benign and malignant lung diseases, and LaparoSonic coagulating shears (Ethicon Endo-Surgery, Inc., Cincinnati, OH, USA) for small thoracotomy. Three-dimensional diagnosis with computed tomography is used to make thoracoscopic surgery safer. The diagnosis and treatment of ground glass opacity of the lung, which is considered to indicate early adenocarcinoma, are being evaluated.

Many basic research studies are also underway. In the morphological expression-related advancement of the molecular genetic analysis of lung cancer, we are investigating whether carcinogenesis of the lung, as reflected by carbohydrate antigen 19–9 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.

We are now developing an Internet video-viewing system that will provide better circumstances for education and research about surgery.

*Breast and endocrinology surgery*

Our group is focusing on the research to link treatment strategies. Recently, it has become apparent that breast cancer is divided into various types, and, thus, a more individualized approach is needed. Multicenter studies involving DNA microarray analysis are under way to find effective drugs for individual tumors. Possible treatment strategies for triple-negative breast cancer have also been investigated. Sentinel lymph-node navigation surgery has been a standard procedure to detect lymph-node metastases in patients with breast cancer. However, the use of sentinel lymph-node biopsy after preoperative chemotherapy remains controversial. Therefore, the feasibility of sentinel lymph-node biopsy after preoperative chemotherapy has been evaluated. Hormone therapy with an aromatase inhibitor is the first choice of treatment for postmenopausal patients with hormone-receptor—positive breast cancer; however, the severe side effects, such as osteoporosis, must be considered. We are investigating the effect of aromatase inhibitors on bone mineral density and the effectiveness of therapeutic options in cases of decreased bone mineral density in postmenopausal Japanese women with breast cancer. Various antihormonal agents have been used against hormone-receptor—positive breast cancer. A multicenter study of a new antihormonal agent has been performed in patients with hormone-receptor—positive metastatic breast cancer refractory to previous antihormonal therapies. A diagnosis of cancer causes patients emotional distress. We are collaborating with a psychiatrist to assess the psychological effect of an initial diagnosis of breast cancer and changes over time.

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# Department of Surgery
# Division of Pediatric Surgery and Vascular Surgery

Takao Ohki, *Professor and Chairperson*
Yuji Kanaoka, *Assistant Professor*
Joji Yoshizawa, *Assistant Professor*
Atsushi Ishida, *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 the endovascular repair of aneurysms, the treatment of peripheral arterial disease with drug-eluting stents, and the clinical study of specific antibodies for heparin-platelet factor 4 (PF4) complexes.

## Research Activities

*Education*

Education for medical students: Children undergoing surgery often have congenital anomalies. Therefore, lectures on pediatric surgery for students are based on embryology.

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

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

*Clinical studies*

1. Endoscopic treatment for vesicoureteral reflux using Deflux

There are 3 options for treating vesicoureteral reflux. We select endoscopic treatment with a dextranomer/hyaluronic acid gel (Deflux, Q-Med, Uppsala, Sweden). We have treated 3 cases, 2 of which completely resolved.

2. Electrolyte and acid-base balances in laparoscopic surgery

Carbon oxide alters electrolyte and acid-base balances in laparoscopic surgery.

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

is performed. This procedure is performed laparoscopically at our hospital. With minimally invasive laparoscopic surgery, pain is minimized, and postoperative recovery is faster. The number of neurologically handicapped children with gastroesophageal reflux has been increasing at our hospital.

4. The Nuss procedure aims to force the sternum forward and hold it there with an implanted steel bar, but without 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 at our hospital is the third highest in Japan.

*Basic studies*

1. Laparosopic surgery contributes to global warming

Carbon dioxide, the most important greenhouse gas, is indispensable for laparoscopic surgery. To assess $CO_2$ emissions, we first determined the number of laparoscopic operations performed in Japan. Next, we measured the quantity of $CO_2$ used in our hospital.

2. Inhibitory effects of an antiangiogenesis drug on the metastasis of human neuroblastoma

Many antiangiogenesis factors have been discovered. We evaluated the effects of several potent antiangiogenesis drugs on the metastasis of neuroblastoma in a mouse model of liver metastasis.

3. Plasmapheresis in severe sepsis or septic shock

During sepsis, microorganisms release various endotoxins that activate, to a greater or lesser extent, cascade systems, including the release of 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 effect of plasmapheresis.

*Vascular surgery*

1) Development of endovascular repair of thoracoabdominal aneurysms

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

2) Research on drug-eluting stents in the superficial femoral artery

The Zilver PTX Drug-Eluting Peripheral Stent is specifically designed and approved to

treat peripheral arterial disease affecting the superficial femoral artery, which is the main blood vessel in the thigh. It is a self-expanding stent made of nitinol, a space-age, shape memory metal that offers unique mechanical advantages for a stent in the superficial femoral artery.

Both the global registry and the randomized controlled trial, which enrolled patients predominantly in the United States, but also in Germany and Japan, is awaiting its 1-year primary endpoint, which should be complete in August 2009. We are participating in this randomized controlled trial.

3) Clinical study of specific antibodies for heparin-PF4 complexes

Heparin is commonly used for anticoagulation in vascular surgery. Heparin-induced thrombocytopenia is a rare but life-threatening complication that causes 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 heparin-induced thrombocytopenia. The prevalence of these antibodies in patients receiving heparin is presumably underestimated. Accordingly, we prospectively measured antibodies against heparin-PF4 complexes and the activity of PF4 and investigated whether they are related to symptoms of heparin-induced thrombocytopenia, particularly in patients undergoing major vascular surgery. We measured these variables in about 300 patients for 2 years.

Antibodies against heparin-PF4 complexes were found in approximately 13% of patients, a percentage higher than expected. Moreover, the antibody-positive patients tended to have higher PF4 activity than did antibody-negative patients. The results of this study are being statistically analyzed and will be published in 2009.

4) Research on hemostatic fleece and closure devices in endovascular aortic aneurysm repair

We have found that collagen patches coated with components of fibrin glue significantly reduce blood loss and the time required for hemostasis at the operation site in endovascular aortic aneurysm repair. Moreover, percutaneous aortic aneurysm repair with closure devices has been shown to be technically feasible and to be associated with a low morbidity rate. However, complications from percutaneous arterial closure are not insignificant and can be life-threatening. We have evaluated our experiences with this technique, compared them with previously published results, and identified factors associated with complications and conversion to open repair.

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# Department of Orthopaedic Surgery

Keishi Marumo, *Professor*
Takuya Otani, *Associate Professor*
Makoto Kubota, *Assistant Professor*
Mamoru Yoshida, *Assistant Professor*
Yutaka Ueno, *Assistant Professor*
Kazuo Asanuma, *Associate Professor*
Hiroki Funasaki, *Assistant Professor*
Fumiaki Masui, *Assistant Professor*
Mitsuru Saito, *Assistant Professor*

## General Summary

*Basic research*

The research carried out in our department, from basic studies on connective tissue cells to application of their results in clinical settings, has been highly appraised in the scientific world, both in Japan and other countries. Moreover, our research has been published in many English-language journals and has constantly received competitive scientific grants. The amount of academic funds awarded has markedly increased during this academic year, and 8 large scientific grants have been awarded to our researchers during the last 5 years.

*Clinical research*

The validity of our clinical projects, strongly backed by basic research and carried out in cooperation with many academic institutions, has been well established, to such a degree that comments related to our studies have been published in *Nature*-related journals. As a result, foreign scientists have further evaluated our findings, and, therefore, the validity of our research has become even more evident. Hence, a fundamental truth cultivated in our department has been proven correct: "an orthopaedic surgeon, a specialist dealing with bones, cartilages, ligaments, tendons, vessels and skin shall validate his/her everyday clinical concerns through a basic research". From now on and with the above truth in mind, the department's vision is to progress into the well-established direction and produce research findings that are original to our department, first in the world, and in accordance with international academic standards.

## Research Activities

*A new plate system for the treatment of proximal humeral fractures*

We introduced a new plate system for proximal humeral fractures and reviewed the outcomes of 7 patients who underwent procedures using the new system. In all patients bone union was obtained without displacement or necrosis, although a varus deformity developed after surgery in one patient. The new system resolves problems related to the treatment of anatomical fractures and of 3-part fractures with a broken great tuberosity.

*A novel pedicle screw for osteoporotic spine*

We designed a new screw with side holes for polymethylmetacrylate augmentation for treatment of patients with osteoporosis and performed a pullout test to evaluate its

efficacy in lumbar vertebrae obtained from fresh cadavers with osteoporosis. The mean pullout force of the designed screw was 853.4 N (1.74 times higher than that of conventional screws). The results show that the newly designed screw is useful for polymethylmetacrylate augmentation in patients with osteoporosis.

*Femoral detorsion osteotomy for osteoarthritis due to developmental dysplasia of the hip in adults*

Although femoral detorsion osteotomy is indicated for the treatment of developmental dysplasia of the hip (DDH) in children, its usefulness for osteoarthritis due to DDH in adults is not known. We performed femoral detorsion osteotomy in combination with acetabular procedures, such as rotational osteotomy, Chiari osteotomy, and the shelf operation, in 8 patients with DDH. Good short-term results were obtained both clinically and radiographically in all patients. The morphological and biomechanical advantages of femoral detorsion osteotomy include decreasing the femoral neck-shaft angle, increasing the femoral head offset, medialization/descent of the femoral head location, and lateralization of the greater trochanter. All of these factors can increase centripetal force and the stability of the hip joint.

*Patient-specific templating technique in total knee arthroplasty*

In our department, we have performed various analyses using computer-assisted surgery in total knee arthroplasty and have been developing a total knee arthroplasty system that uses a patient-specific templating method based on advanced computer graphic technology. The accuracy of component positioning is being evaluated. A computer navigation system in total knee arthroplasty allows proper positioning of the femoral and tibial components, but the complex registration process, lengthening of the operation time, increased cost, the steep learning curve, and the exposure to radiation remain as disadvantages. We have developed an approach in which patient-tailored knee guides may eliminate some of these shortcomings and allow correct placement of components of the artificial knee joint.

*Development of osteotomy plates for hallux valgus*

Hypermobility of the first ray has recently been identified a predisposing factor in hallux valgus deformity. According to our previous studies, in hallux valgus deformity the first ray deviates dorsomedially during weight-bearing movements, and both longitudinal and transverse arches become flat. To correct this deformity, 3-dimensional osteotomy of the first metatarsal is necessary. Starting last year, we have been developing specialized osteotomy-locking plates. Their configuration and size variations, directions, number, and osteotomy angles were examined. With the development of the osteotomy-locking plates, any type of hallux valgus operation can be performed with the same method, and improvements in postoperative results can be expected.

*Collagen cross-links in aging and as a cause of bone fragility in osteoporosis and diabetes mellitus*

Collagen cross-linking, a major posttranslational modification of collagen, plays an

important role in maintaining the biological and biomechanical features of bone. Our recent studies emphasize the important aspects of bone collagen cross-linking with regard to aging and bone fragility in osteoporosis and diabetes. Recent basic and clinical studies of collagen cross-links have entered a new era. For example, on the basis of the results of our basic research *in vitro* and *in vivo,* measurements of levels of pentosidine in serum and urine are now being used to predict fracture risk in patients with osteoporosis and diabetes.

*Clinical outcomes of giant cell bone tumors of the radius*

We studied clinical outcomes of giant cell bone tumors of the distal radius. The examined cases were all grade 3 according to the Campanacci classification. After aggressive curettage and adjuvant therapy with 99% ethanol, iliac bone grafting with plates and external fixation was performed. Recurrence and osteoarthritis were observed in 1 case. An average score of Enneking's limb function test was 80%. This surgical method is simple, allows good local control, and, thus, is a useful initial treatment for giant bone tumors of the radial bone.

*Generation of bone in vivo for revision surgery using beta-tricalcium phosphate and bone morphogenetic protein 2*

The objective of this study is to develop a new technique to generate a large quantity of transplantable osseous tissue near the joints, and investigate whether this tissue may be used to repair segmental bone defects in a rabbit model. A bone-growth factor, recombinant human bone morphogenetic protein 2, and $\beta$-tricalcium phosphate were implanted to the bone tissue near the joint. New bone tissue developed and was then implanted in a bone defect, which healed with cortical bone within 12 weeks after implantation. Our results suggest that this technique is minimally invasive and may be useful for the surgical revision of joints.

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# Department of Neurosurgery

Toshiaki Abe, *Professor*
Shizuo Oi, *Professor*
Satoshi Tani, *Professor*
Hisashi Onoue, *Associate Professor*
Yuzuru Hasegawa, *Assistant Professor*
Tatsuhiro Joki, *Assistant Professor*
Haruo Sakai, *Professor*
Yuichi Murayama, *Professor*
Satoshi Ikeuchi, *Associate Professor*
Masato Nakajima, *Assistant Professor*
Yoshiaki Miyazaki, *Assistant Professor*
Yasuko Kusaka, *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

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.

*Development of a new endovascular opening system*
We performed several clinical and basic research studies regarding endovascular therapy.

*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, robotic digital subtraction angiography system, Zeego by Siemens, 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.

*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.

*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.

*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 therapy 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. In our study we try to use doxorubicine within 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 effect of immunotherapy with fusions of dendritic and glioma cells with interleukin (IL)-12 in patients with malignant glioma. The subjects were 15 patients with malignant glioma, ranging in age from 40 to 62 years. 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 with IL-12 at intervals of 3 weeks. Fusion cells were injected subcutaneously close to a cervical lymph node, and IL-12 was injected transvenously. There were no serious adverse effects, and partial responses have been observed in 2 patients.

*Neurotrauma*

Traumatic acute subdural hematomas in the Japan Neurotrauma Data Bank were categorized into a focal brain injury group and a diffuse brain injury group and were analyzed to clarify the pathophysiological and therapeutic aspects of these injuries. The pathophysiological and therapeutic aspects of acute subdural hematoma associated with diffuse brain injury appear to differ from those with focal brain injury alone.

During the past decade neurobiochemical markers of brain damage have attracted increasing attention in neurotraumatology. The aim of this study was to investigate S-100B protein and neuron-specific enolase (NSE) as serum markers of brain cell damage after traumatic brain injury. Venous blood samples for measurement of S-100B protein and NSE were obtained after admission and the following day. Serum levels of S-100 protein and NSE were compared with the Glasgow Coma Scale score, computed tomographic findings, and outcome after 3 months. Serum concentration and kinetics of S-100B protein and NSE allow the clinical assessment of primary brain damage and have predictive value for outcomes after traumatic brain injury.

*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

We have been measuring the pressure volume index and out-flow resistance to reveal cerebrospinal fluid (CSF) blockage, before and after surgery. The aim of this study is to determine the proper surgical procedure prior to the operation.

2. Electrophysiological research in patients with syringomyelia

The goal of the surgical treatment of syringomyelia is to collapse the syrinx. However, even after this goal has been achieved, some patients still have intractable pain. This pain, which is thought to be caused by damage to the dorsal horn of the spinal cord, is difficult to relieve. We examined somatosensory evoked potentials with median nerve stimulation to reveal the correlation of pain relief and alterations in somatosensory evoked potentials before and after surgery.

3. 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.

4. Analysis of predictive factors in syringomyelic patients

With the introduction of magnetic resonance imaging and advances in neurosurgical techniques, outcomes of patients with syringomyelia have improved significantly. However, the outcomes are determined not only by the surgical result but also by various preoperative conditions. We are using multivariate statistical analysis to examine predictive factors in patients with syringomyelia.

*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 6 years more than 1,000 new cases of various entities have been collected and recorded in our data bank, including hydrocephalus (27% of cases), spina bifida (25%), brain tumors (13%), and craniofacial anomalies (8%). Since April 2003, clinical research fellows, 12 from other domestic universities and 8 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 (J Neurosurg 106: 2006). 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 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 cyst (J Neurosurg: 103, 2005). We have been collecting the largest series of patients and have developed other related new instruments (J Neurosurg 106: 2006).

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, the Kawabuchi Award in 2005, the Bhagwati Oration in 2006, and the Joon-Ki Kang Lecture in 2006.

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. Our department has continued as the headquarters of the International Study Group on Neuroendoscopy, the Japanese Society for Pediatric Neurosurgery, the Japan Academy of Hydrocephalus Research, and a member of our department has served as the President of the Executive Board Committee of the International Society for Pediatric Neurosur-

gery and the Japan Association of Medical English Education.

*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, new devices for anterior cervical interbody fusion and cervical laminoplasty have been developed, and their efficacy has been proven. The Artis Zeego system (Siemens), newly installed in operating room 5, with a navigational system, offers one of the most sophisticated and unique image-guided surgery systems in the world.

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

*Hypothalamopituitary disease*

The endoscopic biendonasal transethmosphenoidal approach, a new surgical technique for sellar and parasellar diseases which we developed, was established as an approved operative procedure. With this method, we use only an endoscope without any nasal speculum. We have performed more than 170 operations and have proceeded with the introduction of a navigation system for nasal surgery and special surgical instruments. We are now trying to find a safer route of approach.

Numerous therapeutic drugs for hormone-producing pituitary adenomas are being developed. The standard treatment for prolactinomas is pharmacotherapy with bromocriptine, terguride, and cabergoline, but definitive criteria for treatment selection have not been established. We studied the relation between the results of drug-loading tests and therapeutic effects, performed a long-term analysis of many cases, and studied the problems of pharmacotherapy. These studies should prove useful for establishing criteria for treatment selection in pharmacotherapy for prolactinoma.

We investigated the mechanism of action of a somatostatin analogue for growth-hormone-producing pituitary adenoma and clarified one part of the mechanism of action.

Furthermore, we are investigating ACTH precursors that are produced by subclinical ACTHomas.

**Publications**

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***Takao H, Murayama Y, Ebara M, Ishibashi T, Saguchi T, Irie K,Yoshioka H, Mori Y, Vinuela F, Abe T.*** New thermoreversible liquid embolic agent for embolotherapy: technicalreport. *Neuroradiology* 2009; **51:** 95–8.

***Terao T, Yokochi F, Kawasaki T, Okiyama R, Hamada T, Nishikawa N, Izawa N, Shin M, Kumada S, Takahashi H.*** Microelectrode findings and topographic reorganisation of kinaesthetic cells after gamma knife thalamotomy. *Acta Neurochir* 2008; **150:** 823–7.

***Tanaka A, Abe T.*** Therapeutic efficacy of STI-571, c-kit tyrosine kinase inhibitor, used in combination with TNP-470, an angiogenesis inhibitor, in the treatment of glioma. *Jikeikai Med J* 2008; **55:** 1–6.

***Shimizu J, Matsumoto M, Yamazaki E, Yasue M.***

Spontaneous regression of an asymptomatic meningioma associated with discontinuation of progesterone agonist administration: case report. *Neurol Med Chir* 2008; **48:** 227–30.

***Kajiwara I, Tanaka T, Kan I, Ohtsuka T, Sawauchi S, Murakami S, Abe T.*** Intracystic hematoma of middle fossa arachinoid cyst caused by ruputured of iinternal carotid-posterior communicating artery aneurysm. *Neurol Med Chir* 2008; **48:** 220–2.

***Akiyama M, Tateshima S, Hasegawa Y.*** Cortical laminar necrosis caused by critically increased intracranial pressure in infant: case report. *Neurol Med Chir* 2008; **48:** 326–9.

***Ishibashi T, Mirayama Y, Urashima M, Saguchi T, Ebara M, Arakawa H, Irie K, Takao H, Abe T.*** Unruptured inracrania aneurysms incidence of rupture and risk factors. *Stroke* 2009; **40:** 313–6.

# Department of Plastic and Reconstructive Surgery

Mitsuru Uchida, *Professor*
Kunitoshi Ninomiya, *Associate Professor*
Shintaro Matsuura, *Assistant Professor*
Kimihiro Nojima, *Assistant Professor*
Meisei Takeishi, *Associate Professor*
Takeshi Miyawaki, *Associate Professor*
Yoko Kishi, *Assistant Professor*

## General Summary

Research in the Department of Plastic and Reconstructive Surgery is focused on 4 basic areas: 1) the etiology and treatment of craniofacial anomalies, 2) the etiology 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 the department consists of surgeons representing virtually all areas of plastic surgery and clinicians from related disciplines. This diversity provides a stimulating atmosphere necessary for productive research. The participation of plastic surgery residents and postresidency fellows in research studies provides 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 the peptides derived from mice with a mutation of Pro 253 Arg have been successfully prepared.

*Gene transfer into limb bud using electroporation technique*

Electroporation was used to transfer genes into the extremities of cultured mammalian embryos. Ell Std-ddy mice were anesthetized with ether. Embryos, together with the placenta and embryonic membranes, were dissected from the surrounding decidua. The yolk sac, amnion, and chorioallantoic placenta were preserved in Hank's Balanced solution. An injection of 0.1 $\mu$l of pEGFP-N1 vector was made into the yolk sac. The extremity was grasped with forceps-type electrodes and electroporated with 3 pulses of 30 to 50 V for 50 microseconds. After the amnion had been removed, the embryo was placed in a bottle filled with mouse serum solution. Ninety-five percent $O_2$ and 5% $NO_2$ were supplied to the bottle via a tube 4 times a day. The embryo was cultured at 37°C and rotated 30 times per minute for 24 hours. The placenta was removed, and the embryo was fixed in 4% paraformaldehyde. With liquid nitrogen, frozen sections were prepared and observed with fluorescent microscopy. With 50-V pulses, green fluores-

cent protein was observed throughout the entire embryo but was observed in a more restricted area with 40-V and 30-V pulses. In embryos electroporated with 30-V pulses gene transfer was localized to the epidermis and dermis.

*Distraction osteogenesis*

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

*Morphologic study of bone conduction mechanisms*

Experiments of artificial bone osteoconductivity have involved the extremities more often than the cranium. For this reason we performed an experimental study of osteoconductivity of $\beta$-tricalcium phosphate ($\beta$-TCP) in a cranial bone defect. Bone regeneration was evaluated in full-thickness circular defects (10 mm in diameter) created bilaterally in the parietal bones of adult female Japanese white rabbits. The rabbits were divided into 3 groups. In group A, a 9.5-mm-diameter, 2.0-mm-thick $\beta$-TCP disk was inserted into the bone defect. In group B, a 0.1-g granule of $\beta$-TCP was inserted. In group C, nothing was inserted. The periosteum was repaired, and care was taken to avoid damage to the dura. Bone regeneration was assessed with macroscopic examination, roentgenometry, strength, and histological examination. The results show that $\beta$-TCP has good biocompatibility with cranial bone.

*Tissue engineering*

Flaps lined with mucosa are in great demand for nasal, oral, tracheal, and urogenital reconstruction. Fascia lined by mucosal tissue has 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. The fascial tissue was removed with the muscular tissue 1 week after transplantation. The specimens were stained with hematoxylin and eosin, and immunohistochemical staining for cytokeratin, a specific marker of mucosal cells, was performed. The growth of mucosal tissue was confirmed histologically. Fasciomucosal complex tissue had developed. Fascia proved to be a useful scaffold that cross-links between the transplanted mucosa and the muscle.

*Hemodynamic analysis of capillary blood vessels in diabetic patients*

The increased prevalence of diabetes has increased the prevalence of diabetic foot gangrene. Below-knee or above-knee amputation should be avoided for as long as possible by using both conservative and surgical treatment. However, few effective methods that can be used to predict diabetic foot lesion have been reported except for the ankle-arm pressure index and the cardio-ankle vascular index. We found that video-microscopic hemodynamic analysis of blood flow through capillary vessels in the

pedal eponychium in patients with diabetes can indicate the stage of microangiopathy and may predict diabetic foot lesions. This new device will be used to investigate the effectiveness of prophylactic treatment with an HT2A receptor antagonist.

### Publications

***Mori K.*** Experimental study on creating a mucosa-lined muscle and fascia: a future model for tissue engineered bladder (in Japanese). *Tokyo Jikeikai Ikadaigaku Zasshi* 2008; **123:** 7–14.

***Shoji H, Hirakawa M, Kobayashi M.*** A case of ectopic extramammary Paget's disease affecting upper abdomen. *J Jpn PRS* 2008; **28:** 34–7.

***Suzuki F, Makino Y, Ishida M, Kurihara K.*** Giant adult malignant sacrococcygeal teratoma. *J Jpn PRS* 2008; **28:** 82–7.

***Sano S, Nojima K, Mori K, Uchida M, Kurihara K.*** Examination of urachal anomaly. *J Jpn PRS* 2008; **28:** 225–30.

***Kojima M, Terao Y, Bando M, Kurihara K.*** Investigation and statistical analysis of surgical treatment for metastatic skin cancer. *J Jpn PRS* 2008; **28:** 554–60.

# Department of Cardiovascular Surgery

Kazuhiro Hashimoto, *Professor*
Yoshimasa Sakamoto, *Associate Professor*
Yuzuru Nakamura, *Associate Professor*
Keno Mashiko, *Associate Professor*
Kei Tanaka, *Assistant Professor*
Ryuichi Nagahori, *Assistant Professor*
Kiyozo Morita, *Professor*
Kouji Kawahito, *Associate Professor*
Tatsuumi Sasaki, *Associate Professor*
Hiromitsu Takakura, *Assistant Professor*
Kouji Nomura, *Assistant Professor*
Yoshimasa Uno, *Assistant Professor*

## General Summary

The main activities in our department involved clinical study, evaluation of alterations in cardiac performance and long-term results after corrective surgeries, and experimental study to address clinical problems we are facing. Clinical investigations, including follow-up studies, of valvular and ischemic heart diseases are a focus of our clinical research activities, as are studies of complex congenital anomalies. The recently increased incidence of aortic aneurysm has become another concern in our field. New treatment approaches applying new surgical techniques, new devices, and research outcomes have been investigated and attempted. We also are performing several experimental studies with in vivo models. The experimental projects involve protection of the lung during extracorporeal circulation and postischemic conditioning after cardiac arrest. The major activities are described below.

## Research Activities

*Basic research*

*I* Studies of myocardial protection during open-heart surgery

1. Experimental study of a new strategy for myocardial protection against ischemia/reperfusion injury

1) Preventing reperfusion injury and ensuring prompt functional recovery after prolonged global ischemia in piglet hearts with a high-dose phosphodiesterase III inhibitor at controlled reperfusion with blood cardioplegic arrest

In addition to having inotropic and vasodilatory effects, phosphodiesterase (PDE) III inhibitors protect against ischemia and reperfusion. However, these drugs, particularly during the early reperfusion phase, have yet to be assessed in clinically relevant in vivo models. We examined the effects of high-dose olprinone, a PDE III inhibitor, with terminal warm-blood cardioplegia (TWBCP) with respect to reversing myocardial reperfusion injury.

Fifteen piglets were placed on cardiopulmonary bypass (CPB) and subjected to 90 minutes of global ischemia, followed by 30 minutes of reperfusion. The animals were divided in 3 groups. In group I, the control group, TWBCP was not supplemented; in group II, TWBCP was supplemented; and in group III, TWBCP was supplemented with olprinone. Myocardial performance was evaluated before and after CPB, after which total electrical cardioversion after reperfusion was measured. Levels of troponin-T and

lipid peroxide were measured. Concentrations of olprinone in TWBCP, serum, and myocardial tissue were measured.
Group III with olprinone showed significant performance recovery (81.9±24.5%; $p<0.01$ vs. groups I and II) without electrical cardioversion. Levels of troponin-T and lipid peroxide in group III were lower than those in group I or II ($p<0.01$ vs. group I; $p<0.05$ vs. group II). Olprinone concentrations in group III were within the range of 200 ng/ml doses having negligible inotropic and dilatory effects. We conclude that TWBCP supplemented with high-dose olprinone reduces myocardial ischemia/reperfusion injury by reducing oxidant-mediated lipid peroxidation and that olprinone has myocardial protective effects.
2) Effect of postconditioning: Experimental study using an in vivo piglet model for cardiovascular surgical on reversal of myocardial stunning by ischemic postconditioning
This study tested the hypothesis that ischemia/reperfusion—induced myocardial damage can be reduced by ischemic postconditioning during the early phase of reperfusion. Eighteen piglets with a mean weight of 10.3±1.5 kg underwent 90 minutes of ischemia with single-dose crystalloid cardioplegia followed by 60 minutes of reperfusion on CPB. In 12 of the piglets, the 2 ischemic postconditioning strategies—6 cycles of 10 seconds of ischemia/reperfusion (PC-I) or 3 cycles of 30 seconds of ischemia/reperfusion (PC-II)—were applied before aortic unclamping, whereas the other 6 piglets were not treated (control). Left ventricular (LV) function (systolic/diastolic) was evaluated with end-systolic elastance (Ees) and the LV time constant for pressure decay during isovolumic relaxation (Tau). Myocardial and blood levels of lipid peroxide, troponin-T, and creatine kinase were measured.
Both systolic and diastolic LV dysfunction (depressed Ees: 54±14% of preischemic value, and increased Tau: 240%±38%), associated with oxidants induced biochemical injury (increased creatine kinase, T-troponin, and lipid peroxidates), were noted after 90 minutes of cardioplegic ischemia followed by untreated reperfusion in the control group. In contrast, postconditioning, especially with protocol II, allowed significantly better LV functional recovery (% Ees: PC-I, 67%±23%; protocol II, 130±43%*; *$p<0.01$ vs. control group, 54%±14%. % Tau: PC-I, 140%±60%*; PC-II, 123%±43%*; $p<0.01$ vs. control group, 240%±38%), and less myocardial biochemical injury (myocardial lipid peroxide: PC-I, 123±21%*; PC-II, 134±12%*; $p<0.05$ vs. control group, 180±34%). Also serum levels of creatine kinase, troponin, and lipid peroxide were decreased in the both postconditioning groups.
Ischemic postconditioning during the early phase of reperfusion produced prompt myocardial functional recovery with decreased biochemical injury in an in vivo piglet CPB model. The interval and duration of repeated brief ischemia/reperfusion during postconditioning might be crucial to determine the beneficial effects of ischemic postconditioning.

*Pediatric heart surgery*

1. Fontan operation

1) Long-term results of the lateral tunnel Fontan procedure with autologous tissue
A review of clinical records and data of patients who had undergone staged

univentricular repair, including the bidirectional Glenn (BDG) procedure and the Fontan procedure, demonstrated excellent long-term results (10-year postoperative survival rate=96.7%) after a lateral tunnel Fontan procedure with autologous tissue.

2) Coagulability and fibrinolytic function in Fontan circulation: Possibility of the conversion of anticoagulation therapy

There is still no consensus regarding the postoperative use and duration of warfarin adminstration after the Fontan procedure. Recently, we have evaluated changes in coagulability and fibrinolytic function after surgery and then modified the anticoagulation therapy for patients to normalize coagulability and fibrinolytic function. We have measured plasma levels of thrombin antithrombin-3 complex (TAT) as the index of coagulability and $\alpha$2-plasmin inhibitor-plasmin complex (PIC) as the index of fibrinolytic function in 20 patients who had undergone the extracardiac Fontan procedure (mean age at operation, 4.2 years) without complications. In all patients, intracardiac thrombus was also detected with primarily transthoracic echocardiography during the period of this study. The mean follow-up duration was 18.7 months (range, 6 to 60 months). No late deaths or thromboembolism occurred in these patients. Levels of both TAT and PIC remained higher than normal for 6 months after surgery, even in patients receiving warfarin. The values then began to gradually decline and had almost completely normalized by 12 months. Confirming these results, we have changed anticoagulation therapy from warfarin to antiplatelet agents for such cases. After this change, plasma levels of TAT and PIC have remained lower, and no patient showed thromboembolic event in echocardiography.

This study suggests that Fontan patients might need warfarin for anticoagulation therapy for the first year after surgery, because of their activated status of coagulability. However, warfarin could be replaced by an antiplatelet agent for patients who show normal results, and no major complications have occurred for 12 months after surgery. We are also reminded that further evaluation and follow-up are important and necessary.

3) Intraoperative evaluation of pulmonary flow reserve capacity and a new method for predicting post-Fontan hemodynamic status

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

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

Surgical outcomes and long-term results were reviewed, with a focus on autograft durability, in 35 patients who had undergone the Ross procedure from 1995 through

2008 with total aortic root replacement and pulmonary autografting. Autograft function was assessed with periodic echocardiographic evaluations for up to 14 years after the operation. There was no operative or acute deaths or late reoperations for autograft regurgitation in 3 patients: (% freedom from reoperation for autograft failure, 87% over 14 years). Excellent durability of the implanted pulmonary autograft valve was noted, especially in children and in patients with preoperative aortic stenosis.

*Adult cardiac surgery*

1. Ten-year results of aortic valve replacement with the Carpentier-Edwards pericardial bioprosthesis: Consideration of patient-prosthesis mismatch

From June 1996 through March 2008, 244 patients underwent aortic valve replacement with a Carpentier-Edwards pericardial valve. Their mean age was 69.8±6.4 years. The patients received a 19-mm valve (n=53), a 21-mm valve (n=87), a 23-mm valve (n=80), or a 25-mm valve (n=24). The survival rate was 91.8% at 5 years and 87.2% at 10 years. The rate of freedom from valve-related death was 98.2% at 10 years. The patients with patient-prosthesis mismatch, as indicated by an indexed effective orifice area (IEOA) less than 0.85 $cm^2/m^2$, did not have poorer outcomes. Moreover, the mean pressure gradient on postoperative echocardiography in these patients was greater than 10 mmHg. We conclude that the hazard point of an IEOA of 0.85 $cm^2/m^2$ for patient-prosthesis mismatch should be reconsidered.

2. Electron beam cine computed tomography—based evaluation of left atrial function after the maze procedure for mitral valve regurgitation

There has been little study of whether atrial function is equally restored by surgery in patients with mitral regurgitation and atrial fibrillation and in patients with mitral regurgitation and sinus rhythm.

We measured atrial volume with electron beam tomography, which has excellent temporal resolution and minimizes motion artifacts, and used the data to construct left atrial volume-time curves. The subjects were 33 patients with or without atrial fibrillation who had undergone surgery for mitral regurgitation and 11 control patients.

In patients with sinus rhythm, left atrial volume decreased significantly, regurgitation resolved soon after surgery, and the reserve function was well maintained. Left atrial booster pump function was also well maintained before and after surgery. In patients with atrial fibrillation that resolved after maze surgery, the left atrial volume was larger immediately after surgery than that in patients who had sinus rhythm and did not improve in the postoperative period. These patients had lower reserve function and much lower booster pump function despite restoration of sinus rhythm. Patients with mitral regurgitation and atrial fibrillation that spontaneously reverted to sinus rhythm after valve surgery without the maze procedure showed intermediate values for left atrial function.

The maze procedure is unlikely to restore atrial function in patients with mitral regurgitation and atrial fibrillation, even if sinus rhythm returns postoperatively. Because postoperative left atrial function in patients with sinus rhythm was similar to that in control patients, surgery should be considered for patients with severe mitral regurgitation while atrial function and sinus rhythm are maintained.

3. Effect of preoperative LV percent fibrosis on midterm outcomes after aortic valve replacement

The aim of this study was to investigate the effect of preoperative LV fibrosis on midterm outcomes after aortic valve replacement (AVR) and the relation between the plasma brain natriuretic peptide (BNP) concentration after AVR and the preoperative percent fibrosis.

Sixteen patients who underwent single AVR and left ventricular endomyocardial biopsy at the operation were enrolled in this study. The mean age at operation was 52.9±18.2 years. The total follow-up period was 118 patient-years with a mean follow-up period of 7.4±0.6 years. Serial echocardiographic examinations were performed before and after surgery in all patients. On the day of the final follow-up echocardiographic examination, the plasma BNP concentration was measured in 10 patients.

The preoperative percent LV fibrosis was 26.0%±9.2% (range, 10.8 % to 46.2%). The LV mass index (LVMI) decreased significantly from 201±92 to 143±79 g/m² and returned to the normal range after surgery in 9 of 16 patients. The LVMI in these 9 patients was less than 200 g/m² before the operation. The degree of preoperative LV fibrosis differed significantly between patients in whom the LVMI did (22.6%±7.0%) and did not (30.4%±10.3%, p=0.0456) normalize after surgery. The preoperative percent LV fibrosis was also strongly correlated with both the preoperative and postoperative LVMIs. This strong correlation indicated that the regression of LV hypertrophy after surgery depends on the progression of preoperative LV fibrosis, which is a decisive morphological alteration in LV remodeling. The BNP concentration 7.4±0.6 years after surgery was also strongly correlation with the preoperative percent LV fibrosis, being higher in patients with severe fibrosis.

A preoperative LVMI less than 200 g/m² can be a reliable predicator of reversible LV remodeling after valve replacement and should be taken into account when surgical intervention is considered.

## Publications


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# Department of Obstetrics and Gynecology

Tadao Tanaka, *Professor*
Kazuhiko Ochiai, *Professor*
Takekazu Onda, *Professor*
Seiji Isonishi, *Associate Professor*
Aikou Okamoto, *Associate Professor*
Kyousuke Yamada, *Assistant Professor*
Hirokuni Takano, *Assistant Professor*
Hideo Shinozaki, *Assistant Professor*
Kazunori Ochiai, *Professor*
Hiroshi Sasaki, *Professor*
Naoki Kamiya, *Professor*
Shigeki Niimi, *Associate Professor*
Shigemitu Kobayashi, *Assistant Professor*
Kuniaki Ohura, *Assistant Professor*
Satoshi Takakura, *Assistant Professor*
Kentaro Sugiura, *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. Integrated Copy Number and Expression Analysis of Chemoresistant Ovarian Carcinomas

Women with serous ovarian cancer are often intrinsically refractory to platinum-taxol—based treatment or become resistant on relapse. Because accurately predicting the response to chemotherapy remains possible, we sought to identify somatic DNA copy number variation (CNV) associated with primary resistance in advanced-stage disease. Genome-wide frequency and the level of CNV in 118 ovarian tumors were measured with single nucleotide polymorphism microarrays. A well-defined subset of 85 advanced-stage serous tumors was then used to relate CNV to primary resistance to treatment. The discovery-based approach was complemented by quantitative polymerase chain reaction analysis of copy number of 12 candidate genes previously reported to be associated with clinical outcome in ovarian cancer. Likely CNV targets and tumor molecular subtypes were further characterized by gene expression profiling. Amplification of 19q12, containing cyclin E (CCNE1) and 20q11.22-q13.12, mapping immediately adjacent to the steroid receptor co-activator NCOA3, was significantly associated with a poor response to primary treatment. From previously reported associations of copy number with outcome, only the amplification status of CCNE1 was validated as a marker for primary chemoresistance. Chemoresistant tumors with high CCNE1 copy number and protein expression were predictably associated with increased cellular proliferation, as were a subset of treatment-responsive patients, suggesting a cell-cycle—independent role for CCNE1 in modulating chemoresponse. Patients with poor clinical outcomes and without CCNE1 amplification over-expressed genes involved in extracellular matrix deposition. Our findings identify 2 distinct mechanisms of primary treatment failure in serous ovarian cancer, involving CCNE1 amplification and enhan-

ced extracellular matrix deposition.

2. Mesenchymal-to-epithelial transition during the inclusion cyst formation from human ovarian surface epithelium

Most surface epithelial-stromal tumors of the ovary are thought to arise from epithelial inclusion cysts. Thus, these cysts are precursor lesions of ovarian carcinoma. On the basis of this hypothesis, we aimed to characterize the human ovarian surface epithelium in which the mesenchymal-to-epithelial transition occurs in the process of inclusion cyst formation. We used specimens from 9 patients with endometrial cancer who underwent hysterectomy and bilateral salpingo-oophorectomy. Immuohistochemical studies were performed of 10 normal ovaries containing 92 inclusion cysts and 4 normal fallopian tubes to examine the expression of antigen markers, including calretinin, podoplanin, D2-40, thrombomodulin, human bone marrow endothelial (HBME)-1, vimentin, epithelial membrane antigen (EMA), WT1, carbohydrate antigen (CA) 125, MOC31, tumor-associated glycoprotein (TAG) 72, Ber-EP4, and E-cadherin. We found that positive staining rates for mesothelial markers in normal ovarian surface epithelium were 100% (10 of 10) with calretinin, 80% (8 of 10) with podoplanin, 80% (8 of 10) with D2-40, 70% (7 of 10) with thrombomodulin, 100% (10 of 10) with HBME-1, and 100% (10 of 10) with vimentin; that positive staining rates for epithelial markers in tubal epithelium were 100% (4 of 4) with HBME-1, 100% (4 of 4) with vimentin, 100% (4 of 4) with EMA, and 75% (3 of 4) with TAG-72, and 100% (4 of 4) with Ber-EP4; and that positive staining rates for both markers in inclusion cysts were 51.1% (47 of 92) with HBME-1, 44.6% (41 of 92) with vimentin, 65.2% (60 of 92) with TAG-72, and 88.0% (81 of 92) with Ber-EP4. Ovarian surface epithelium has both mesencyhmal and epithelial characteristics. In contrast, inclusion cyst gains more epithelial characteristics with the loss of mesencyhmal characteristics. These findings support a mesenchymal-to-epithelial transition during inclusion cyst formation from ovarian surface epithelium.

3. MicroRNA (miRNA) expression profiles for cancers, including those of the lung, breast, stomach, prostate, and colon, were examined to investigate the miRNA involvement in carcinogenesis. We are now investigating the roles of miRNA in the resistance of human ovarian cancer cells to paclitaxel. Our findings may have significant implications for therapeutic strategies aiming to overcome cancer cell chemoresistance.

4. A randomized trial of retroperitoneal closure versus opening has been registered to prevent lymphedema after lymphedenectomy for patients with uterine cervical cancer or endometrial cancer. A total of 150 cases will be registered; 64 cases had been registered by March 2009.

5. This small proof-of-principle study has demonstrated that there are profiles in the serum of Japanese ovarian cancer patients that can be used to classify the presence of cancer. The information is similar to that in ovarian cancer sera from the United States because N-dimensional clusters built on United States sera spectra created cluster maps predictive of the Japanese samples. The model performed better than any of the existing single biomarker assays, although truly useful models await a much larger sample size and the use of independent validation sample sets to demonstrate their robustness. These results encourage us to start a large-scale, multisite collection of sera from Japanese patients with ovarian cancer to develop a Japanese ovarian cancer serum profile assay.

*Fetomaternal medicine*

1. Antiphospholipid syndrome (APS) is a clinical entity manifested by arterial and venous thromboses and recurrent miscarriages and is caused by antiphospholipid antibodies. Recently, APS has also been observed with some complications of pregnancy, e.g., pregnancy-induced hypertension, intrauterine growth restriction, and late fetal death. However, little is known about how APS is involved in these complications. The Fc receptor for IgG (Fc$\gamma$ receptor) is implicated in some autoimmune diseases. To investigate the pathological significance of the Fc$\gamma$ receptor in APS and complications of pregnancy, we have attempted to establish an experimental model for APS using Fc$\gamma$ receptor knock-out mice.

We examined the presence of antiphospholipid antibodies in patients who had obstetrical complications, and investigated placental pathology.

2. Many patients with recurrent pregnancy loss become infertile or have repeated spontaneous abortions after infertility therapy. These transitional conditions have not been researched so far. We have investigated the different possible causes and clinical manifestations of these conditions from the perspective of reproductive failure.

3. Establishment of an immortalized human extravillous trophoblast cell line by retroviral infection of E6/E7/human telomerase reverse transcriptase

Investigation into the function of human trophoblasts has been restricted by a lack of suitable cell models. We aimed to obtain long-lived human normal trophoblast cell lines that would serve as ideal *in vitro* cell models. Primary human trophoblast cells were derived from the placenta of a woman who had undergone elective abortion during the 7th week of gestation. The cells were immortalized by infection with retroviral expression vectors containing type 16 human papillomavirus E6 and E7 in combination with human telomerase reverse transcriptase (hTERT). Characterization of the cell line was performed. Immunocytochemical staining for human chorionic gonadotrophin chain $\beta$, cytokeratin 7, human leukocyte antigen G, and CD9 indicated an extravillous trophoblastic phenotype. Transwell insert invasion assay showed the invasiveness of this cell line, and gelatin zymography showed secretion of matrix metalloproteinases 2 and 9. Karyotype analysis showed almost normal chromosomal number with small deviations ranging from 46 to 48, and a nude mouse assay showed no tumorigenecity. This newly immortalized cell line, HChEpC1b, will provide a useful model for the study of extravillous trophoblast function.

4. Dynamics of biopyrrins in pregnant women and newborns

Biopyrrins are bilirubin oxidative metabolites that can be measured in pregnant women to monitor psychological stress. Urinary biopyrrin levels were significantly elevated by delivery from $4.22\pm0.47$ to $7.33\pm0.68$ (U/g•Cre). Induction of delivery increased postpartum biopyrrin levels by 89% ($10.74\pm1.37$ vs $5.67\pm0.80$ (Ug•Cre). These data suggest that pregnancy and delivery increase psychological stress and that delivery induction increases stress even more.

*Reproductive endocrinology*

1. Most of the mechanisms for achieving pregnancy have been clarified owing to advances in assisted reproductive technology. Nevertheless, the mechanism of implanta-

tion remains unclear.

CD147 is expressed at high levels on cell surfaces of various tumors and stimulates matrix metalloproteinases. We hypothesized that CD147 may play an important role in implantation. The aim of this study was to determine the expression and hormonal regulation of the CD147 gene during the human implantation period in controlled ovarian hyperstimulation cycles. We found that levels of CD147 and matrix metalloproteinase 2 mRNA in human endometrium were significantly decreased during the secretory phase in controlled ovarian hyperstimulation cycles.

## Publications


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# Department of Urology

Shin Egawa, *Professor*
Isaho Ikemoto, *Associate Professor*
Hiroshi Kiyota, *Associate Professor*
Nozomu Furuta, *Assistant Professor*
Takashi Hatano, *Assistant Professor*
Shoichi Onodera, *Professor*
Koichi Kishimoto, *Associate Professor*
Koji Asano, *Associate Professor*
Yasuyuki Suzuki, *Assistant Professor*
Kenta Miki, *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 projects to clarify the biology of urological malignancies and develop new therapeutic tools. Most projects were reported at the annual meeting of Japanese Urological Association and the American Urological Association. The projects are as follows:

1) Proteomic analysis of new biomarkers for prostate cancer and urothelial cancer
2) Gene therapy for urological malignancies
3) Establishment and biological analysis of a new prostate cancer model derived from Japanese patients
4) Research on prostate cancer stem cells
5) Basic research in neurourology and female urology

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

1) Study of seeds and hormones for intermediate-risk prostate cancer
2) Clinical study of high dose rate brachytherapy with external beam radiation therapy for high-risk prostate cancer

*Genitourinary tract infection*

1. Basic research on the antimicrobial resistance of *Neissseria gonorrhoeae,* especially resistance mechanisms in cephem-resistant *N. gonorrhoeae*
2. Clinical surveillance of gonococcal urethritis in the Tokyo metropolitan area
3. Clinical study of additive therapy with clarithromycin and cefteram for male gonococcal urethritis
4. Psychological education for chronic prostatitis/chronic pelvic pain syndrome

*Neurourology and urodynamics*

1. Study of dysuria

Clinical assessment of elderly patients with dysuria treated with low-dose medications to avoid problematic side effects

Efforts were made to advertise the importance of the frequency-volume chart.

2. Study of nocturia

The relation between cardiac insufficiency and nocturia was demonstrated by means of brain natriuretic protein and the frequency-volume chart.

We started a study of the relation between dyssomnias and dysuria using the International Prostate Symptom Score and the Pittsburgh Sleep Quality Index.

*Kidney and adrenal gland*

1. Clinical study of postoperative steroid replacement for preclinical Cushing syndrome
2. Clinical study of the effectiveness of adrenal venous sampling for primary aldosteronism
3. Laparoscopic surgery for adrenal tumors

*Endourology and extracorporeal shockwave therapy*

1. Extracorporeal shockwave therapy for Peyronie disease
2. Holmium-yttrium-aluminum-garnet laser ablation of the prostate for benign prostatic hypertrophy

**Publications**

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**Reviews**

***Miki J, Rhim JS.*** Review prostate cell cultures as *in vitro* models for the study of Normal stem cells and cancer stem sells. *Prostate Cancer Dis* 2008; **11:** 32-29.

# Department of Ophthalmology

Hiroshi Tsuneoka, *Director and Professor*
Keigo Shikishima, *Associate Professor*
Genichiro Takahashi, *Associate Professor*
Kazushige Toda, *Associate Professor*
Tadashi Nakano, *Assistant Professor*
Kenichi Kohzaki, *Assistant Professor*
Takaaki Hayashi, *Assistant Professor*
Takuya Shiba, *Assistant Professor*
Osamu Taniuchi, *Professor*
Hisato Gunji, *Associate Professor*
Satoshi Nakadomari, *Associate Professor*
Masaki Yoshida, *Assistant Professor*
Akira Watanabe, *Assistant Professor*
Tsutomu Sakai, *Assistant Professor*
Katsuya Mitooka, *Assistant Professor*
Koichi Kumegawa, *Assistant Professor*

## General Summary

The main research interest of our department is the pathophysiology of the visual processing system. The following topics are the subjects of basic and clinical studies: color vision, ocular oncology, histopathology, biochemistry, eye movement, neuro-ophthalmology, corneal and refractive surgery, cataract, glaucoma, electrophysiology, diabetes, vitreoretinal diseases, and uveitis.

## Research Activities

*Color vision defects and genetic analysis of retinal diseases*

1. We performed genetic testing of 26 male subjects with X-linked red/green color-vision deficiencies. The genotypes of the L and M visual pigment genes were determined with the polymerase chain reaction. Diagnoses of 11 protans and 15 deutans were made. All 13 dichromats had no difference in absorption maxima, whereas 85% (11 of 13 subjects) of anomalous trichromats had some separation in absorption maxima. However, no distinctive genotypes were found to distinguish severe (dichromacy and severe anomalous trichromacy) from mild (mild anomalous trichromacy) forms. Our results suggest that genetic testing is beneficial for distinguishing dichromacy from anomalous trichromacy.
2. We performed clinical and molecular genetic analysis of various inherited retinal diseases, such as retinitis pigmentosa and macular and cone dystrophies. We identified causative mutations in these diseases. To clarify disease haplotypes, results of haplotype analysis with 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, which is a common cause of blindness in the elderly in industrialized countries. More than 500,5688 single nucleotide polymorphisms of the entire genome were genotyped with Affymetrix Human Mapping Arrays and TaqMan assay. We are now analyzing the data to determine which single nucleotide polymorphisms are involved in Japanese patients with age-related macular degeneration.

*Ocular oncology and histopathology*

1. Ocular findings associated with systemic malignant tumors were reviewed. The clinical features of metastasis to the orbit and the choroid and of adnexal and ocular

malignant lymphoma and paraneoplastic syndrome were explained in detail. The preoperative evaluation, operative techniques, and management of complications in orbitotomy were described in a book.
2. We reported an unusual case of malignant solitary fibrous tumor arising in the orbit.

*Biochemistry*
1. We investigated the protective effect of pigment epithelium—derived factor-loaded nanoparticles (PEDF-NPs) against photoreceptor degeneration in P23H transgenic rats. Immunocytochemistry did not show greater opsin preservation in PEDF-NP—treated retinas than in blank NP-treated retinas but did show a significantly higher number of photoreceptors. The results suggest that intravitreal injection of PEDF-NPs does not prevent photoreceptor degeneration in the P23H rat retina.
2. We evaluated the effects of intravenously administered methoxy polyethylene glycol-(D, L-lactide) (PLA-PEG) (stealth) nanoparticles (NPs) encapsulating betamethasone phosphate (BP) on experimental autoimmune uveoretinitis (EAU) in Lewis rats. The cy7-stealth NPs accumulated in the retina and choroid of rats with EAU and remained during the subsequent 3-day period. Furthermore, systemically administered BP-stealth NPs reduced in 1 day the clinical scores of rats with EAU, which were maintained for 2 weeks, and decreased the histological scores. In addition, the expression of inflammatory cytokines was reduced with this treatment. In conclusion, systemically administered BP-stealth NPs inhibit the development of EAU due to the targeting and the sustained release of steroids.

*Eye movement*
Binocular summation on the visual cortex was explored by comparing cortical responses of binocular and monocular visual stimulation by means of functional magnetic resonance imaging (fMRI). Two different checkerboard stimuli were used. The binocular condition demonstrated signal intensities markedly higher than those in the monocular condition. Two stimuli demonstrated different binocular summation ratios and different increasing signal intensities. These results suggest that the binocular summation processes of these 2 visual stimuli differ in the visual cortex.

*Neuro-ophthalmology*
1. Endothelin (ET)-1 is a potent vasoconstrictor peptide, and increased ET-1 levels have been described in diseases associated with vascular dysregulation. The pathogenesis of nonarteritic anterior ischemic optic neuropathy (NAION) is poorly understood but may involve vascular dysregulation and vasoconstriction of the nutrient vessels supplying the optic nerve head. To clarify the possible pathophysiological role of ET-1 in the development of NAION, we examined plasma ET-1 levels in patients with NAION. We found increased plasma ET-1 levels in patients with NAION. Our data indicate that elevated plasma ET-1 may be an important risk factor in the development of NAION and suggest that an ET-receptor antagonist might offer a new therapeutic approach to this disease.
2. The assessment of the pupillary reflex in patients with diabetes and the diagnosis of

ocular torticollis were reviewed. Articles on the treatment for neuroretinitis, the differential diagnosis of anisocoria, and the surgical technique of blepharoptosis were published. Neuro-ophthalmological examination in the fields of otorhinolaryngology and neurosurgery was described in books. Brief outlines of neuro-ophthalmology and general ophthalmology were included in textbooks for students of medicine and pharmacology.

3. We reported a case of optic neuropathy with severe visual loss that was detected during the follow-up of a patient with systemic lupus erythematosus. Early corticosteroid pulse therapy was effective in the treatment of severe optic neuropathy associated with systemic lupus erythematosus. We reported the features of optical coherence tomography of retinal lesions in neuro-ophthalmologic disorders and the clinical findings of anti-aquaporin 4 antibody-positive optic neuritis.

*Cornea and refractive surgery*

The cornea group at The Jikei University chooses the most appropriate method of corneal surgery by discussing the various options with each patient. We replace only those corneal components that are actually damaged. Photorefractive keratectomy is effective for cases of surface corneal opacity, and automated lamellar therapeutic keratoplasty (ALTK) is also effective for cases of corneal stroma opacity. We also perform Descemet's stripping automated endothelial keratoplasty for corneal endothelium dysfunction.

We have adapted several new treatments for all diseases, such as dry eye, corneal infection, corneal injury, hereditary corneal disease, allergic corneal disease, and keratoconus. ALTK, in which a microkeratome is used to make a lamellar flap, was performed in several cases of corneal opacity. We found that ALTK allows earlier suture removal and induces less astigmatism than does conventional lamellar keratoplasty.

We studied the clinical outcomes of secondary implantation of iris-clip intraocular lens for aphakic eyes for 5 years postoperatively. Clinically significant complications were not found with specular microscopy or a laser flaremeter.

*Glaucoma*

1. We compared the effectiveness of Humphrey Matrix perimetry, scanning laser polarimetry with variable corneal compensation (Zeiss GDx scanning laser polarimeter), and optical coherence tomography (Zeiss Stratus OCT 3000) for the early detection of glaucoma. We found that the detection precision of Humphrey Matrix perimetry was equal to those of these optical imaging devices and that pattern standard deviation was the most effective variable.

2. We evaluated the shape and structure of blebs after the trabeculectomy from various angles. We examined risk factors causing leaking bleb and overhanging bleb in relation to operative method and postoperative management.

3. We investigated the effect of ocular optical system aberrations in perimetry, especially for peripheral vision measurement. In peripheral vision, there were astigmatism and coma aberrations, and the point images that were projected onto the retina differed

with the measurement site. These findings suggest that the aberration affects the results of threshold measurements in the peripheral vision.

*Electrophysiology*

We are performing electroretinography (ERG) to evaluate functional retinal disorders in hereditary retinal degeneration diseases.

The ERG waveforms are recorded as response waves from retinal cells, such as ganglion, amacrine, bipolar, and photoreceptor cells. On the ERG, we use 3 types of machines to obtain full-field, multifocal, and color ERGs. The full-field ERG is recorded according to an international standard, and the responses from cone and rod cells can be separated. The multifocal ERG reflects mainly cone function in the central 30-degree area of the retina which shows mainly cone function, and can record 61 elements. The color ERG records responses separately from long-, middle-, and short-wavelength cones.

In the future, we will evaluate waveforms recorded from full-field ERG, which can be more fully analyzed with a personal computer program. Moreover, as we extract a waveform from a single type of retinal cell, we will be able to investigate retinal disorders at a more detailed level.

*Diabetes and vitreoretinal diseases*

We have used 23-gauge and 25-gauge transconjunctival vitrectomy systems for 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 intraocular lens (Eternity, Santen Pharmaceutical Co., Ltd.) for combined pars plana vitrectomy, phacoemulsification, and intraocular lens implantation, we observed the visibility of the retina during vitrectomy and measured the depth of anterior chamber preoperatively and postoperatively with the Pentacam (Oculus Optikgeräte GmbH).

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

*Uveitis*

1. A novel therapy with a chimeric antibody against tumor necrosis factor alfa for Behçet disease

Intravenous infliximab significantly decreased the frequency of ocular attacks and improved visual acuity. In addition, we found that intraocular surgery can be performed effectively and safely to improve the vision of patients receiving infliximab therapy for Behçet disease.

2. A case of ocular toxocariasis with neuroretinitis

Ocular toxocariasis is usually associated with a history of *Toxocara canis* infection and causes various ophthalmic manifestations, including chronic endophthalmitis, posterior pole granuloma, and peripheral granulomatous inflammatory mass. Neuroretinitis is a rare manifestation of ocular toxocariasis. Oral prednisolone was effective in inhibiting local inflammatory and immunologic responses.

*Visual neuropsychology*

1. Two temporal channels in human V1 identified with fMRI

We measured responses to spatial uniform (Ganzfeld) luminance changes in the human visual cortex by means of fMRI. We attempted various temporal modulation stimuli without a spatial contrast pattern and found that the blood oxygen level—dependent signal consisted of transient and sustained channel responses that had been reported in previous psychophysical studies. By identifying these 2 independent channels with linear model analysis, we revealed that the relative contribution of these channels varies with the eccentricities across V1.

2. The objective visual field map with fMRI

We developed a software program to analyze visual field maps obtained with fMRI. To evaluate the usefulness of this analysis, we planned an experiment with hemifield visual stimuli and succeeded in drawing a pseudohemianopsic visual field map.

3. Neural plasticity of area V1

We used fMRI to assess abnormal V1 signals in patients with juvenile macular degeneration. These signals have been interpreted as indicating cortical plasticity. Subjects viewed a stimulus passively or performed a stimulus-judgment task. During passive viewing, there were large unresponsive V1 regions. We refer to these regions as the lesion-projection zones. In patients with juvenile macular degeneration, we observed highly significant responses in the lesion-projection zones while they performed a task. These task-dependent signals can be explained by hypotheses that have very different implications for V1 plasticity. We propose that these responses were driven by feed-back signals by the task demands. Deletion of retinal feed-forward inputs may unmask pre-existing task-dependent feed-back signals that are ordinarily suppressed.

*Low vision*

We used two methods to measure the visual acuity of patients with low vision and brain injury and examined the degree of divergence. Each eye's visual acuity was measured with both Teller Acuity Cards and Landolt Rings. We further examined the degree of visual field loss. The visual acuity measured with Teller Acuity Cards was significantly better than that measured with Landolt Rings for patients with brain injury and eccentric viewing.

We reported on children of school age who visited the low-vision clinic at Kanagawa Rehabilitation Hospital and on those who were supported by a home for visual disabilities.

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# Department of Otorhinolaryngology

Hiroshi Moriyama, *Professor*
Takakuni Kato, *Professor*
Hiromi Kojima, *Associate Professor*
Makoto Iida, *Assistant Professor*
Mamoru Yoshikawa, *Assistant Professor*
Minoru Iida, *Assistant Professor*
Yuji Umezawa, *Professor*
Atsushi Hatano, *Associate Professor*
Nobuyoshi Otori, *Associate Professor*
Yasuhiro Tanaka, *Assistant Professor*
Yoshinori Matsuwaki, *Assistant Professor*

## General Summary

Our basic and clinical studies have examined: the pathogenesis of cholesteatoma and adhesive otitis media, surgery of the middle ear, navigation medicine, space motion sickness, nasal allergy, endoscopic endonasal sinus surgery, sleep apnea syndrome, phonosurgery, deglutition, and reconstructive surgery for head and neck tumors.

## Research Activities

*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 improve the safety of surgery. In addition, we recorded cases of cholesteatoma surgery conducted at our hospital in our database, which is used to analyze the clinical condition of patients, consider operative methods, and review postoperative outcomes. In regard to research on hearing loss, we are studying the physiology of the inner ear in metabolic disorders using experimental animal models and collaborating with Shinshu University in the gene analysis of deaf patients.

Approximately 200 middle ear surgeries are performed annually at our hospital. The several cochlear implantations performed every year have also yielded favorable results. We conduct skull base surgery, including surgery for cholesteatoma in the petrous part, in conjunction with the department of neurosurgery, and have found that hearing and facial nerve function can be preserved in many cases. We also conduct acoustic tumor surgery via the posterior cranial fossa approach, middle cranial fossa approach, or translabyrinthine approach.

For secretory otitis media, we select the treatment method on the basis of the degree of development of the mastoid air cells. We determine the timing of the removal of indwelling ventilatory tubes in each patient by measuring changes in middle ear total pressure caused by transmucosal gas exchange.

In the field of neuro-otology, we have introduced vestibular evoked myogenic potential (VEMP) testing for the evaluation of saccular function in patients with such conditions as vestibular neuritis, Meniere's disease, and dizziness of unknown cause, to facilitate detailed diagnosis and treatment. Moreover, we are examining the prevalence of abnormal saccules, as measured with VEMP testing, in the ictal and nonictal phase of

Meniere's disease and the incidence of VEMP abnormalities according to disease stage. We also perform furosemide-loading VEMP in patients with suspected delayed endolymphatic hydrops as a putative test for 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 using single photon emission computed tomography in conjunction with the department of neurology.

In the selection of astronauts for the Japan Aerospace Exploration Agency, our neuro-otology team conducted the third-stage examination at the Tsukuba Space Center. In this examination, the aptitude for space flight was tested by applying "Coriolis stimulation" using a rotating chair to provoke motion sickness.

*Rhinology*

We have been analyzing data from patients undergoing endoscopic sinus surgery (ESS) for rhinosinusitis and from prospective studies of the postoperative course to identify factors related to refractory disease. In an attempt to expand the indications of ESS from paranasal sinus tumors to skull base surgery, including for spinal fluid leakage, skull base tumors, and pituitary gland tumors, and to improve the safety of ESS, we have conducted high-tech navigation surgery in which 3-dimensional endoscopic images and stereonavigation images are displayed in a superimposed manner, and we have identified problems and improvements relevant to this operative method. At present, we are making alterations to the device to improve accuracy and performance. We have examined the involvement of aspartate protease derived from fungi, especially from *Alternaria,* and the superantigen of *Staphylococcus aureus,* in the pathogenesis of refractory eosinophilic paranasal sinusitis. Through comprehensive gene expression analysis to clarify factors contributing to intractable chronic sinusitis, we have found that the expression profiles of genes related to virus infections differ between fibroblasts derived from cell cultures of nasal polyps and those derived from normal tissue cultures. At present, we are studying the regulatory mechanisms of gene expression to clarify the mechanisms underlying the differential gene expression.

*Head and neck tumors*

We perform radical surgery for common advanced cancers (*e.g.,* total pharyngolaryngectomy combined with reconstruction by free intestinal transplantation for hypopharyngeal cancer and total laryngectomy for laryngeal cancer); however, we actively undertake laryngeal conservation surgery (partial hypopharyngectomy combined with reconstruction by free flap and partial laryngectomy) as functional preservation treatments, especially to preserve vocal functions to the greatest extent possible, which has yielded favorable outcomes, from the aspects of both laryngeal preservation and survival. As conservative therapy and postoperative treatment for advanced cancer, concurrent chemoradiotherapy with cisplatin and fluoruracil or radiotherapy or both are performed and have yielded 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 mesopharyngeal and hypopharyngeal superficial cancers.

In research on cancer, we are performing fundamental studies to apply basic findings to future studies or clinical practice, and such fundamental studies include extraction of DNA from surgical specimens and evaluation of epidermal growth factor receptor expression, a target for molecularly targeted agents. We are planning clinical studies of human papilloma virus expression, which is thought to be involved in the development of mesopharyngeal cancer and oral cancer, and are planning to investigate treatments for various cancers, including vaccine therapy.

*Vocal and swallowing functions*

1. Phonosurgery: We are conducting outpatient day surgery using a flexible fiberoptic scope and 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.
We have been performing outpatient day surgery for unilateral recurrent nerve paralysis by intravocal fold injection of atelocollagen for many years; however, we are also performing laryngeal framework surgery for patients who are not considered candidates for intravocal fold injection of atelocollagen.
2. Diagnosis and treatment for spasmodic dysphonia: We have been performing botulinum toxin treatment as first-line therapy for spasmodic dysphonia with the approval of the ethics committee of the university since December 2004. The prevalence of this disorder is increasing; therefore, evaluating methods of diagnosis and treatment is important, and an important future task is the development of 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 engage in teamwork with co-medical staff, such as nurses. We consider therapeutic strategies for clinical conditions by evaluating patients using videoendoscopy and videofluorography tests and are promoting training for swallowing.

*Sleep apnea syndrome*

We have attempted to construct a system that can deal with patients from various clinical fields besides otorhinolaryngology, including psychiatry, respiratory medicine, cardiovascular internal medicine, pediatrics, and dentistry, and with visiting medical officers. However, because the number of patients visiting our hospital is increasing, novel approaches are required. Thus, we are planning to provide remote medical care and to perform examinations for sleep disorders using "telesomnology," which is an applied version of an information technology topic covered by the Japanese Society of Sleep Research, starting this year. The clinical research items covered as research concepts include: 1) nasal breathing and the stability of sleep, 2) sleep disturbance associated with allergic rhinitis (pollen allergy), 3) attention-defict hyperactivity disorder—like symptoms in children with obstructive sleep apnea syndrome (OSAS), 4) physical development of children with OSAS, 5) maxillofacial growth and sleep-disordered

breathing in children with the adenoid facies, 6) a new surgical treatment for adult OSAS, integrating knowledge from many clinical departments, and 7) the development of telesomnology.

Sleep has been found to have significant associations with otorhinolaryngologic diseases, such as allergic rhinitis and gastroesophageal reflux disease.

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# Department of Anesthesiology

Shoichi Uezono, *Professor*
Sachiko Omi, *Professor*
Ichiro Kondo, *Associate Professor*
Masaki Kitahara, *Assistant Professor*
Chieko Fujiwara, *Assistant Professor*
Kazuhiro Shoji, *Assistant Professor*
Takehiko Nezu, *Professor*
Masanori Takinami, *Associate Professor*
Yasushi Mio, *Associate Professor*
Yoshie Taniguchi, *Assistant Professor*
Shigehiko Uchino, *Assistant Professor*

## General Summary

The 2008 academic year is the fourth year that the Department of Anesthesiology has been under the leadership of Professor Shoichi Uezono. The functions of the Department of Anesthesiology are innovative, high-quality patient care; teaching; and research into perioperative medicine, intensive care medicine, and comprehensive pain management. In the past year we made progress and accomplished much with the support of our faculty, the institutional administration, and the Dean of The Jikei University. Below we highlight some of our research achievements of the past year.

## Research Activities

Research continues as an increasingly important part of our department's activities. This past year the department had 11 active clinical protocols. As another index of research activity, 12 abstracts were accepted for presentation at this year's meeting of the Japanese Society of Anesthesiologists. In addition, several abstracts were accepted and presented at international meetings, including highly competitive meetings, such as the annual meeting of the American Society of Anesthesiologists. Members of our department have continued to be invited as visiting professors and guest speakers at national and international meetings. Listed below are the ongoing research projects in which the principal investigators were members of the Department of Anesthesiology.

Clinical studies were performed in all areas of the department, including the main operating room, the intensive care unit (ICU), and the comprehensive pain clinic. These clinical studies included a wide variety of investigative efforts. Dr. Fujiwara and her colleagues examined the usefulness of the pleth variability index, an index automatically derived from pulse oximeter waveform analysis, to optimize perioperative fluid status. Dr. Shoji's group continued to study whether postoperative use of dexmedetomidine infusion enhances epidural pain medication. Dr. Hirabayashi examined whether herbal medicine administered the night before surgery reduces preoperative anxiety. Dr. Shibasaki assessed the usefulness of 3 mL of 2% lidocaine as a test dose in epidural anesthesia. Dr. Taniguchi has been interested in temperature regulation during surgery and its effects on postoperative outcomes in patients undergoing head and neck surgery. Dr. Uezono has been an active investigator of the mechanisms of difficult airway in children, particularly the association between micrognathia and neural crest cells. The ICU staff remain active in clinical research including: 1) the study of

outcomes of ICU patients who have undergone tracheostomy (Dr. Kase); 2) international observational study of acute kidney injury in ICU patients (Dr. Uchino); 3) analysis of risk factors for acute postoperative kidney injury in patients with massive intraoperative bleeding (Dr. Uchino); 4) application of citrate as an anticoagulant during hemodialysis (Dr. Uchino); and 5) multicenter study of hemoperfusion with a polymyxin B fiber column in patients with sepsis (Dr. Kase). Physicians in the pain clinic are also involved in clinical research. Dr. Kitahara continued his efforts to establish gold standards of objective pain assessment. He is also assessing the effect of tramadol on various types of chronic pain. Dr. Kojima has been an active member of the national cancer research council on postmastectomy pain syndrome. Some of these clinical studies received grants from sponsoring companies.

Basic science investigations included studies of gene therapy for experimental pulmonary hypertension (Dr. Uezono), studies of the effects of various anesthetics on the release of substance P in the dorsal horn of the spinal cord after tissue injury (Dr. Kondo), the development of new methods of endotoxin measurement (Dr. Kase), mechanisms of anesthetic postconditioning in myocardial mitochondria (Dr. Mio), and mechanisms of anesthesia-induced neural excitation (Dr. Yasui). A Grant-in-aid for Scientific Research (*kakenhi*) was successfully renewed during the past year for Dr. Uezono, and Dr. Yasui was awarded a new Grant-in-aid for Young Investigators.

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

Masahiro Abo, *Professor*
Takeshi Kamikubo, *Assistant Professor*
Kazushige Kobayashi, *Associate Professor*
Keiji Hashimoto, *Assistant Professor*

## General Summary

The main research projects in our department have focused on perfusion-weighted imaging, diffusion-weighted imaging, brain injury, falls, the easy Z-score imaging system, voxel-based stereotactic extraction estimation, dysphagia, gastrostomy catheters, and stroke rehabilitation.

## Research Activities

Perfusion-weighted imaging and diffusion-weighted imaging: Although the perfusion-weighted imaging/diffusion-weighted imaging mismatch model has been proposed to identify patients with acute stroke who would benefit from reperfusion therapy, the optimal definition of a mismatch is uncertain. We evaluated the odds ratio for a favorable clinical response to reperfusion compared with no reperfusion in patients at various mismatch ratio thresholds enrolled in the Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution study. A mismatch ratio of 2.6 provided the highest sensitivity (90%) and specificity (83%) for identifying patients in whom reperfusion was associated with a favorable response. Defining mismatch with a larger weighted imaging/diffusion-weighted imaging ratio may provide greater power for detecting the beneficial effects of reperfusion.

National Institutes of Health Stroke Scale: For patients with stroke and a perfusion-weighted imaging /diffusion-weighted imaging mismatch treated with intravenous tissue plasminogen activator at 3 to 6 hours, a substantial change in the baseline National Institutes of Health Stroke Scale (NIHSS) score ($\geq$10 points) is a potent discriminator of patients who experience early reperfusion from those who do not. In addition, an NIHSS score of $\leq$2 appears to be an excellent endpoint for phase II studies of reperfusion therapies.

Patients with traumatic brain injury: To investigate brain activation in the prefrontal cortex during the Wisconsin Card Sorting Test (Keio Version) (KWCST), we examined changes in total hemoglobin volume in 8 patients with traumatic brain injury (TBI) and 20 healthy control subjects using 2-channel near-infrared spectroscopy. We found that mean total hemoglobin volume in the right prefrontal cortex during the KWCST in patients with TBI ($-0.131\pm0.127$) was significantly lower than that in control subjects ($0.016\pm0.135$; $2\times3$ ANOVA; $p<0.05$). These results demonstrate that patients with TBI have lower circulation of hemoglobin in the right prefrontal cortex during the KWCST than do control subjects.

Dysphagia: The factors affecting the risk of accidents during the replacement of gastrostomy catheters remain unknown and, therefore, have not been thoroughly investigated.

We performed a nationwide questionnaire survey of 415 rehabilitation-training facilities for the replacement of gastrostomy catheters. We received 221 valid responses. Among the catheter-replacement methods submitted, the bumper button replacement method was the most widely chosen, comprising 40% of the valid responses. The measures used to prevent accidental erroneous catheterization included examination of the stomach contents, endoscopic examination of the stomach, and the detection of insufflation sounds, although these measures varied widely among the facilities. Fifty-one of the 221 facilities that responded to the survey reported various mishaps, of which 20 were due to erroneous catheterization. In approximately 40% of the facilities, there was no operative manual for the replacement nor was the patient's consent obtained before the procedure was performed. This investigation clarifies the risks involved in the replacement of gastrostomy catheters. This survey also suggests that the methods for catheter replacement should be re-examined to prevent accidents.

Preventing Falls: Falls and fall-related injuries, such as hip fracture, are among the most common medical problems of older persons. Predisposing factor for falls can be divided 2 categories, intrinsic factors and extrinsic factors. Intrinsic factors include motor impairments, sensory disturbances, cognitive dysfunctions, and psychological factors. Extrinsic factors include environmental factors and medication use. Of these factors, a history of falling is a consistently proven predictor of the risk of future falls. Patients with stroke, dementia, or Parkinson disease are usually considered to be at high risk for falls. Many randomized controlled trials have examined the effects of preventive interventions, such as physical exercise and environmental modifications, in elderly persons living at home, in care facilities, or in hospitals. These studies have shown that a multifactorial intervention program can prevent falls. The strategy for fall prevention comprises an exercise program for improving motor function and environmental modifications. For persons living at home, regular physical exercise, consisting of muscle training, balance training, and aerobic exercise, is recommended as "preventive rehabilitation". For persons living in care facilities and for in-patients, the risk of falls should be assessed at the time of admission. The assessment will indicate intervention needed to reduce the risk of falls. The hospital staff should assess ambulation, transferring, toileting, beds, rooms, floor, stairs, bathrooms, footwear, lighting, and medications and modify them if they are inappropriate for preventing falls. In addition, some physical practice is recommended to prevent disuse syndrome. Physical restraints might be needed for some patients with psychological problems, although restraints should not be introduced easily. Hip protectors are effective for preventing hip fracture due to falls but do not prevent falls themselves. Health professionals should pay more attention to falls prevention.

**Publications**

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# Department of Emergency Medicine

Takeki Ogawa, *Professor*
Joji Ohtsuki, *Associate Professor*
Kei Ohtani, *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 the laboratory assessment of heart attack in the emergency room
5. Managing immediate cardiac life support
6. Providing logistical support to the Japan Boxing Commission

## Research Activities

1. Director of Japan Neurotrauma Data Bank Committee
2. Prognostic value of heart fatty acid binding protein for patients with chest symptoms in the emergency room
3. Research committee on higher cerebral function after traumatic brain injury
4. Research committee on impact biomechanics in automobile accidents (Society of Automotive Engineers of Japan, Inc.)
5. Published a revised edition of *Guidelines for the Treatment and Management of Severe Head Injury* (the Japan Society of Neurotraumatology)
6. Research group on traumatic intracranial hypotension
7. Supervision and development of ultrasound devices for the diagnosis and treatment of cerebrovascular disorders
8. Secretary of research group on surgical maneuver for trauma
9. Management course of Japan Advanced Trauma Evaluation and Care

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***Shimizu K, Koyama T, Harada J, Abe T.*** CT-guided percutaneous laser disk decompression for lumber disk hernia. *IVR: Interv Radiol* 2008; **23:** 41–6.

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# Department of Endoscopy

Hisao Tajiri, *Professsor*
Hiroshi Kakutani, *Associate Professor*
Takeshi Suzuki, *Assistant Professor*
Hiroo Imazu, *Assistant Professor*
Mitsuru Kaise, *Associate Professor*
Tomohiro Kato, *Assistant Professor*
Matsuda Koji, *Assistant Professor*
Muneo Kawamura, *Visiting Professor*

## General Summary

Our main research activities are clinical studies of endoscopic diagnosis and the treatment of gastrointestinal and hepatobiliary-pancreatic diseases. In addition, we performed basic research on the development of novel instruments, image processing and analysis, and optical apparatuses, such as auto fluorescence imaging (AFI), narrow-band imaging (NBI), endocytoscopy, confocal laser endomicroscopy, and high degree of freedom therapeutic endoscopes. Published achievements and recent reports are summarized below.

## Research Activities

*Pharyngeal, esophageal and gastric malignancies*

1. Endoscopic diagnosis for esophagogastric neoplastic lesions

Early detection and accurate diagnosis of premalignant and malignant lesions in the pharynx, esophagus, and stomach are essential for selecting the most appropriate therapeutic strategy for each patient. The following novel optical technologies are used clinically in addition to conventional white-light endoscopy. We have designed a series of prospective clinical studies to evaluate and validate the benefits of the following novel imaging technologies. Most recently we have introduced an ultrathin transnasal endoscope that is expected to improve patient cooperation, especially for screening at a nonreferral hospital by reducing discomfort during endoscopic examination.

1) Magnifying endoscopic observation using a NBI system

This new diagnostic system consists of a magnifying ($\times$90) endoscope and an NBI light source and provides detailed morphological information about capillaries on the mucosal surface. Our current study focus is to develop algorithms for NBI technology that will allow prediction of the histological type of gastric carcinoma and the tumor extent without biopsies and allow early detection of precancerous changes in the specialized columnar epithelium of Barrrett's esophagus. The preliminary achievements have already been reported at several conferences and have been published. We have also introduced our own classification of gastric cancer based on magnified NBI findings and demonstrated its advantages over conventional diagnosis in a prospective study.

2) AFI

The AFI endoscopy system has recently been developed to endoscopically visualize autofluorescence emitted from the wall of the gastrointestinal (GI) tract. Theoretically, AFI may allow detection of premalignancies or early-stage malignancies without a distinct endoscopic appearance. Although AFI is still associated with a high false-

positive rate, we found that AFI, in combination with conventional white-light imaging and NBI, can improve specificity.

3) Ultrathin endoscope (transnasal endoscope)

The ultrathin endoscope can reduce discomfort during the endoscopic examination. However, the image resolution of the ultrathin endoscope is poorer than that of conventional endoscopes, and therefore is associated with an inherent risk of a higher false-negative rate. Indeed, we found that for gastric lesions the false-negative rate was higher than with a high-resolution endoscope. We are trying to develop methods to study motility disorders of the esophagus with an ultrathin endoscope by assessing symptoms during examination. Details of this motility study are described later.

4) Endoscopic ultrasound-guided fine-needle aspiration biopsy

Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) biopsy allows histopathological analysis of endoscopically undetectable lesions within and outside the walls of the GI tract, such as esophageogastric submucosal tumors and mediastinal and abdominal lymph nodes. In EUS-FNA biopsy, the biopsy needle can be precisely guided into the lesions with real-time ultrasonographic imaging. The tissues obtained with EUS-FNA biopsy are immediately examined by a cytologist or pathologist to evaluate the presence of malignant cells. Evaluation of the technical safety and usefulness of this technique is ongoing.

2. Endoscopic treatment of esophageal and gastric malignancies

With recent advances in endoscopic diagnostic techniques and instruments, the indications for endoscopic therapy for early gastric and esophageal carcinomas have been expanding. Research on the following endoscopic therapeutic methods is now under way to standardize them as treatments for upper GI tumors.

1) New indications for endoscopic treatment and endoscopic submucosal dissection

Current indications for endoscopic mucosal dissection (ESD) are limited by the size, depth, and histological type of the lesions. Our recent efforts have been focused on expanding indications for ESD for early gastric cancer based on histopathological analysis. We are evaluating small, poorly differentiated adenocarcinomas without ulceration, well-differentiated adenocarcinomas within 30 mm in size or over ($>$30 mm) confined to the mucosa, and carcinomas with microinvasion into the submucosal layer as new indications for endoscopic mucosal resection (EMR) for gastric cancer. For esophageal cancer, current indications for EMR are epithelial cancer (m1) and cancer partially invading the lamina propria mucosae (m2) with a negligible risk of lymph-node metastasis. New indications being evaluated are mucosal cancer invading the lamina muscularis mucosae (m3) and lesions with slight submucosal invasion within the first third of the submucosal layer (sm1). En bloc resection with ESD is considered necessary to expand the indications for endoscopic treatment. The development of a series of endoscopic knives and long-lasting submucosal fluid successfully reduced the technical difficulty of ESD and the risks of complications. By monitoring intragastric pH after treatment, we also evaluated the effectiveness of acid-suppressive drugs that has been used empirically after endoscopic treatments. A study to evaluate with blood cultures the risk of sepsis and endotoxemia after ESD is underway.

2) Therapeutic interventions employing innovative endoscopy systems

The multibending scope (M-scope) is a new type of endoscope with a higher degree of freedom. We have reported previously that the M-scope is useful for treating tumors of the lesser curvature, greater curvature, posterior wall of the gastric body, and the cardiac region, which are not accessible with a conventional endoscope. Studies using an M-scope with magnifying capability are now under way for more accurate and safer procedures. In addition, clinical studies using a newly developed therapeutic endoscope (R-scope), which has a special mechanism allowing the forceps to move laterally and vertically, in addition to the multibending function, are now proceeding with the goal of advancing the development of endoscopic therapy. We also performing several research studies related to natural orifice transluminal endoscopic surgery, including full-thickness resection, as technologies beyond current endoscopic treatments direct at mucosal diseases only.

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, many unknown factors remain concerning this topic. Therefore, it is imperative to clarify this relation in this department, where endoscopic treatment of gastric cancer is performed on a routine basis. The experiments on this topic, especially on DNA methylation due to *H. pylori* infection, have been performed in cooperation with the Department of Gastroenterology, Toshiba General Hospital. We have also been investigating the roles of inducible nitric oxide synthase (iNOS) in the pathogenesis of *H. pylori*-associated diseases and found that *H. pylori* eradication plays important roles in the repair process of methylated DNA and in the alteration of mucosal methylation during the 5 years after *H. pylori* eradication. The preliminary results have already been reported at several conferences and have been published in Japan and internationally. In addition, we reported that the 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, *i.e.,* Lugol chromoendoscopy, has allowed esophageal cancer to be detected at an early stage and has improved prognoses. However, metachronous or synchronous cancer located in the oropharynx or hypopharynx has become the main factor adversely affecting the prognosis and quality of life of patients with esophageal cancer. Although detecting these cancers at an early stage is absolutely essential, Lugol chromoendoscopy is difficult to perform for these cancers, unlike esophageal cancer, owing to their location. Magnifying endoscopy performed in combination with the NBI system has enabled us to detect these hard-to-find cancers at an early stage without Lugol chromoendoscopy. A multicenter, randomized, controlled study of the clinical value of this new combination endoscopy has been performed.

*Functional disorders of the upper GI tract*

The etiology of gastroesophageal reflux diseases, including nonerosive reflux disease and GI motility disorders, are difficult to identify. It is important to establish methods to

evaluate the hypersensitivity and dysmotility of the GI tract to understand the pathophysiology of these disorders and to treat them.
We developed a new method to evaluate esophageal function using a small-caliber endoscope. We started basic experiments for the analysis of the motility and sensitivity of the esophagus with the aim of applying this technique to clinical practice.

*Diagnosis and treatment of esophagogastric varices*
Recently, we have been involved in studies of the hemodynamics of the portal venous system in patients with esophagogastric varices using color-Doppler endoscopic ultrasonography (CD-EUS); these studies have clarified several factors associated with an increased likelihood of recurrence after endoscopic treatment of esophagogastric varices. When all the factors have been identified, we can expect to be able to predict and prevent early recurrence of varices after treatment. We have also started a study to confirm the factors that aggravate hemorrhagic gastritis and cardiac varices. Studies of CD-EUS are multidirectional. CD-EUS is a highly accurate technique for detecting gastrorenal shunts and can delineate shunt status in detail after the treatment of esophagogastric varices. Therefore, this diagnostic system could be useful for selecting patients with esophagogastric varices who are candidates for interventional radiology and for predicting its effects.

*Enteroscopy and colonoscopy*
1. Diagnostic techniques
Capsule endoscopy is a breakthrough modality that enables the detection of diseases of the small intestine that were unreachable with ordinary endoscope systems. Capsule endoscopy had been performed for more than 1 million cases as of May 2009 and is recommended as a first-line examination for detecting diseases of the small intestine. We introduced a single-balloon enteroscope that allows procedures, such as biopsy and hemostasis, for small intestinal lesions.
Recently the incidence of colonic cancer has markedly increased. In particular, the incidence of colon cancer has increased in Japan. In Europe and the United States several studies have examined the use of capsule endoscopy as a screening examination of the large intestine. In Japan we are examining the use of capsule endoscopy as a screening examination for the large intestine at 6 hospitals.
Accurate preoperative evaluation of tumor invasion is essential for selecting the most appropriate therapeutic strategy for colonic lesions. To improve diagnostic accuracy, we use a magnifying endoscope with NBI and/or AFI technology involving conventional white-light observation.
2. Research on endoscopic interventions
Surgical resection has been performed as the treatment of first choice for large sessile tumors in the colon. Recently endoscopic en bloc resection with ESD, which is the standard treatment for gastric lesions, has been used to treat such colonic lesions. However, endoscopic resection of large intestinal lesions is technically difficult because of the narrow space and the much higher rate of complications, such as perforation and bleeding. Our current efforts are focused on establishing a safe and reliable method for

removing large colonic lesions endoscopically; we have just started to apply the ESD technique. Additionally, an infrared endoscopy system is being used to evaluate high-risk vessels on the ulcer base after ESD to prevent bleeding.

3. Capsule endoscopy as enteroscopy

Capsule endoscopy is a less-invasive endoscopic method that allows the detection of lesions in the small intestine which had been unreachable with traditional push enteroscopy. Recently, especially in Western countries, capsule endoscopy has been recommended as the first-line endoscopic examination for the evaluation and management of obscure GI bleeding. We have performed capsule endoscopy in 109 cases since it became covered by the Japanese health insurance system in April 2007. Our study clarified that capsule endoscopy should be performed as soon as possible after a patient visits the hospital with a complaint of melena. We published our results in scientific journals. We are aiming to improve the diagnostic accuracy of capsule endoscopy in the evaluation of obscure GI bleeding by reconsidering the traditional bowel preparation regimen.

*Pancreatobiliary endoscopy*

1. Diagnosis of biliary and pancreatic diseases

Due to recent introduction of Diagnosis Procedure Combination (DPC), a specialized Japanese insurance system, the establishment of a standard systematic diagnostic algorithm for biliary and pancreatic diseases has become more important than ever. We are comparing the diagnostic accuracy for hepatopancreatic diseases among EUS-FNA, multidetector-row computed tomography, magnetic resonance cholangiopancreatography, and endoscopic retrograde cholangiopancreatography (ERCP). Additionally, we introduced a second-generation contrast medium for ultrasonic imaging in EUS diagnosis.

ERCP is well-established procedure but is associated with the risks of severe, possibly life-threatening complications. We designed a new catheter to reduce unplanned pancreatic injection of contrast medium, which is considered a major cause of post-ERCP pancreatitis.

In the diagnosis of ampullary tumors of the duodenum, we perform detailed characterization of the mucosal surface structures and the magnification of microstructures with NBI to determine whether a lesion is benign or malignant. Also, convex array EUS is performed to evaluate the depth of tumor invasion. On the basis of these findings, the indications for endoscopic papillectomy are determined. Favorable clinical outcomes have been obtained so far.

2. Treatment using endoscopic techniques of pancreatobiliary diseases

EUS-guided celiac plexus block has been performed to control persistent pain because of chronic pancreatitis despite the presence of benign disease. We applied EUS-guided celiac plexus neurolysis with the injection of small amounts of ethanol and are evaluating its feasibility.

We have also started experiments with animals to develop new interventional technologies to establish the local control pancreatic cancer and to diagnose gallbladder neoplasms.

*Palliative care*

Palliative care is attracting more and more attention. Various techniques have been developed to provide the best quality of life for critically or terminally ill patients. Endoscopic procedures may play an important role, especially in supporting food intake. In our department percutaneous endoscopic gastrostomy is performed for patients who cannot maintain sufficient oral intake. Although percutaneous endoscopic enterostomy is conventionally not indicated for patients who have undergone gastric surgery, we have extended the use of this procedure for such patients since 1994 and have investigated its clinical usefulness. Kits developed by us for placing percutaneous endoscopic gastrostomies have reduced the frequency of associated complications. To relieve stenosis attributable to tumors of the digestive tract and bile duct, we perform endoscopic ballooning/bougienage and subsequent metallic stenting. The therapeutic results have been good. To reduce the pain associated with chronic pancreatitis and inoperable pancreatic cancer, we perform transgastric celiac plexus block with EUS. These endoscopic procedures may greatly contribute to the improvement of 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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***Sumiyama K, Tajiri H, Gostout CJ (Mayo Clin Rochester).*** Submucosal endoscopy with mucosal flap safety valve (SEMF) technique: a safe access method into the peritoneal cavity and mediastinum. *Minim Invasive Ther Allied Technol* 2008; **17:** 365–9.

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# Department of Infection Control

Shoichi Onodera, *Professor* Masaki Yoshida, *Assistant Professor*

## Research Activities

*Epidemiological research on sexually transmitted infections*

From 2006 through 2008, a study group of the Ministry of Health, Labour and Welfare, "Research on progress of guidelines for the prevention of specified infections related to sexually transmitted infections," was active with Dr. Onodera as chief investigator. The objectives of the study included research and development on preventing the onset and spread of sexually transmitted infections and on promoting countermeasures against sexually transmitted infections based on the contents of "Guidelines for prevention of specified infections related to sexually transmitted infections," revised in 2006. The main items studied were: 1) epidemiological research on onset trends of sexually transmitted infections, 2) early detection of sexually transmitted infections in young people and trials related to treatment, 3) development of rapid and highly precise test methods for genital herpes and genital warts, and 4) surveillance of drug-resistant gonococci and development of diagnostic and therapeutic methods for gonococcal infections of the throat. The main results are described.

According to a survey of onset trends of sexually transmitted infections, decreasing trends for sexual Chlamydia infection and gonococcal infections have been observed in both men and women in Japan since 2003, but the prevalence of genital herpes and genital warts remains largely unchanged in both men and women. An epidemiological survey of all sexually transmitted infections was performed in model prefectures to verify the fixed-point survey. Prefectures asked to cooperate in the survey were Chiba, Ishikawa, and Hyogo for 3 years from 2006 and Iwate, Ibaragi, and Tokushima for 2 years from 2007. As a result, consistent trends in the trends survey on sexually transmitted infections and the complete survey in this research differed by prefecture and disease, but the highest consistency was found for sexual Chlamydia infection, followed by genital herpes and genital warts. The lowest consistency rate was for gonococcal infections.

As an event aimed at young people, Chlamydia self-testing kits (polymerase chain reaction [PCR] method) were distributed by post for 3 years. During that period, the kits were sent to 6,121 persons, and the cooperation of 1,585 asymptomatic young persons was obtained. The rates of Chlamydia infection were 5% in men and 6% in women. In a sexual behavior questionnaire, more Chlamydia-positive persons than Chlamydia-negative persons replied that condoms were not for preventing infections but for avoiding pregnancy, suggesting that they regularly performed sex without using condoms.

These results showed the need to specify definite standards for fixed points and the methods for the design of fixed-point surveys in the future. In addition to undertaking publicity on prevention and supplying information as measures against sexually transmitted infections in young people, the government must maintain smooth relations with

nongovernmental organizations and medical institutions from testing until medical examinations.

*Clinical research*

1. Clinical study of urosepsis

Patient characteristics and treatment results were examined in 55 adults with urosepsis examined at Kanagawa Prefecture Shiomidai Hospital from 2000 through 2007. Of the patients, 90% were elderly, both men and women, and 93% had underlying diseases, such as chronic renal failure, diabetes, and cerebrovascular disorders. The most common causative bacteria were *Escherichia coli,* and all patients in whom *Pseudomonas aeruginosa* or methicillin-resistant *Staphylococcus aureus* was detected had indwelling urethral catheters. Three patients died, and all of them had septic shock as a complication. In initial treatment, antibacterial agents, such as first- to third-generation cephems, carbapenems, and penicillins combined with beta-lactamase inhibitors, were used. The efficacy rate was 54.5%. An analysis of patients in whom initial treatment was ineffective showed errors in designation of the causative organism and in the selection of antibacterial agents because of an insufficient understanding of pathophysiology in more than half. Insufficient doses and numbers of treatments with antibacterial agents were confirmed in 44% of patients. These results indicate that in the treatment of urosepsis, an understanding of the clinical characteristics of patients and disease severity and the selection, based on this knowledge, of appropriate antibacterial agents at sufficient doses are essential.

2. Clinical study of poor prognostic factors in *P. aeruginosa* bacteremia

Because the mortality rate of *P. aeruginosa* bacteremia is extremely high, investigating prognostic factors and establishing effective treatment are essential. From April 2003 through December 2007 at hospitals affiliated with The Jikei University School of Medicine, age, underlying diseases, antibacterial agents used, and bacterial invasion routes were examined in 89 adults in whom *P. aeruginosa* was isolated in blood cultures. The mortality rate of *P. aeruginosa* bacteremia was 24.7%. The most common underlying disease was leukemia, and the most common invasion route was the urinary tract. As initial treatment, effective antibacterial agents were administered to 65.2% of the 89 patients, but the mortality rate was the same as in patients not given effective antibacterial agents. However, poor prognostic factors were thrombocytopenia, polymicrobial infections, and hypoalbuminemia. In our study, unlike in other studies, administration of appropriate antibacterial agents did not improve outcomes.

3. Clinical study of acquired immunodeficiency syndrome-related lymphomas

Treatment of acquired immunodeficiency syndrome-related lymphomas of ileocecal origin: Acquired immunodeficiency syndrome (AIDS)-related lymphomas show a more progressive course than do lymphomas in patients without AIDS and have a poorer prognosis. At present, a standard treatment has not been established. The significance of combination therapy with highly active antiretroviral treatment (HAART) remains unclear. We have used HAART combination chemotherapy to treat AIDS-related lymphomas of ileocecal origin. No severe adverse drug reactions have occurred, and good therapeutic effects against both the human immunodeficiency virus infection and

the malignant lymphomas were achieved, making long-term remission possible. In the future, it will be necessary to recruit more patients, but our study suggested the usefulness of HAART combination chemotherapy in the treatment of AIDS-related lymphomas. Treatment of AIDS-related esophageal primary lymphomas: AIDS-related lymphomas are aggressive and often refractory. In some of our patients with refractory esophageal primary AIDS-related lymphomas, salvage treatment was ineffective. Because the combination therapy of stavudine, amivudine, and fos-amprenavir with rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone (R-CHOP) was ineffective, it was replaced with rituximab, etopsoide, methylprednisolone, high-dose cytarabine, and cisplatin (R-ESHAP) therapy, which was also ineffective. In such patients, ultra-high-dose chemotherapy together with autologous peripheral stem cell transplantation will be necessary.

*Basic research*

1. A method of measuring minimum inhibitory concentrations of antibacterial agents using tetrazolium

The drug sensitivity test for bacteria (measurement of minimum inhibitory concentration [MIC]) assesses whether bacteria grow in a medium containing an antibacterial agent. The results showed that an incubation time of 18 to 24 hours is required. Therefore, we examined the MIC agreement rate between the standard method and a method in which evaluation was possible in 6 hours (rapid method) using the Tetracolor One reagent (Seikagaku Corp.) that combines a tetrazolium salt and an electron mediator. In the MIC measurement using a microplate, the agreement rate was high between the standard method and the rapid method except for some drugs (cephazolin, cefaclor, and minocycline). Because the MIC can be determined in 6 hours with the rapid method, it is useful for the rapid detection of resistant bacteria.

2. Typing analysis of *P. aeruginosa* in clinical isolates with PCR

Typing analysis of bacteria using molecular biology procedures is essential for testing of in-hospital propagation of resistant bacteria such as *P. aeruginosa* and *Acinetobacter*. We studied typing analysis with PCR to determine if it could be used to analyze clinical isolates of Gram-negative bacteria in our hospital. Nucleic acid was extracted from 17 clinical isolates of *P. aeruginosa* obtained in our hospital in 2008. Typing analyses with PCR and with pulsed-field gel electrophoresis (PFGE) were compared. PCR was performed with 2 primers, BOXAIR and ERIC2. The PCR products were subjected to agarose gel electrophoresis, and electroophoretic patterns were analyzed. With the PFGE method, electrophoresis of the DNA fragment after restriction enzyme Spe I treatment using a GenePath kit (Bio Rad Laboratories) was analyzed with a contour-clamped homogeneous electric field apparatus (CHEF DR2, Bio Rad). The typing analysis of clinical *P. aeruginosa* isolates using the BOXAIR and ERIC2 primers showed basic agreement with the results obtained by PFGE. Typing analysis with PCR is a rapid test that can be completed in 1 day, from extraction of the nucleic acids to electrophoresis, and the cost per strain of bacteria is also low. The identification capacity is slightly lower than that of PFGE, but these results showed that PCR is useful as a rapid and simple analytical method for outbreaks of *P. aeruginosa* infection.

## Publications

***Kato T, Sato F, Horino T, Nakazawa Y, Sakamoto M, Yoshida M, Onodera S, Hiroshi Kiyota H.*** Clinical features of 15 cases treated with linezolid. *Jpn J Chemother* 2008; **56:** 202-5.

# Department of Dentistry

Masashi Sugisaki, *Professor*
Kazuo Ioroi, *Associate Professor*
Katsuhiko Hayashi, *Assistant Professor*
Akihiro Ikai, *Associate Professor*
Shigeru Suzuki, *Assistant Professor*

## General Summary

*Clinical studies of temporomandibular disorders*
We have continued our studies of screening questionnaires and the evaluation of quality of life for patients with temporomandibular disorders (TMDs). We studied clinical questions for drafting guidelines for TMDs.

*Morphological and histological studies of the temporomandibular joint*
We continue to study anatomical and histological examination of temporomandibular joint and articular disk in Mammalia.

*Basic studies of oral mucosal keratinocytes*
We examined the expression and function of trefoil factor 3 in normal oral mucosal keratinocytes *in vitro*. *In-situ* immunohistological expression of nerve growth factor and its receptors TrkA and p75NTR in keratinocytes from normal oral mucosa and oral lichen planus was also studied.

## Research Activities

*Clinical studies of TMDs*
1. Epidemiological study of TMDs in working persons in Tokyo
We have developed a 4-item questionnaire for the screening of patients for TMDs, which showed a sensitivity of 0.746 and a specificity of 0.811.
Purpose: Using this 4-item questionnaire, we screened persons working in the Tokyo metropolitan area for TMDs in 2005 and 2006, to determine the prevalence of TMDs and contributing factors.
Methods: We used the secondary data of the Tokyo Dental Association for both 2006 (412 people, 396 effective analysis subject [96.1%]) and 2007 (795 people, 679 effective analysis subjects [85.4%]).
Results: The prevalence of TMDs in men was 19.5% for those in their 20's, 35.1% for those in their 30's, 27.3% for those in their 40's, 14.3% for those in their 50's, and 3.9% for those in their 60's. The prevalence among women was 32.2% for patients in their 20's, 38.3% for those in their 30's, 23.5% for those in their 40's, and 6.1% for those in their 50's. Multivariate logistic regression analysis revealed that significant factors among persons with TMDs were fatigue (odds ratio = 1.55) in men and depression (odds ratio = 1.37) and fatigue (odds ratio = 1.30) in women.
Conclusions: These results were developed from secondary data; therefore, while direct relations cannot be confirmed, the need to investigate work-time sleep and its association

with the onset of TMD was revealed.

2. Comparison of epidemiologic studies of TMDs using the same screening question

Some epidemiological surveys of TMDs have used different questionnaires whose validity has rarely been reviewed; therefore, comparative study is difficult.

Purpose: To study epidemiological surveys of TMDs which have used the TMD screening questionnaire that was acquired by validity examination.

Methods: Subjects were asked the following question: "Do you have jaw pain when you widely open and close your mouth?"

Results: A 2005 survey of dental diseases by the Ministry of Health, Labour and Welfare showed that the percentages of persons replying "yes" were 3.5% (139 of 3,969) among persons aged 15 to 85 years; 20.0% (130 of 649) among persons aged 18 to 70 years who were employed in Tokyo; 5.8% (29 of 501) among persons aged 40 to 55 years living in Yokote-shi, Akita; and 20.5% (258 of 1,261) among persons aged 12 to 93 years who were patients of general clinics in urban and suburban areas of Tokyo.

Conclusions: This study revealed local differences and subject differences in epidemiological survey findings of disease with the same questionnaire. The prevalence of TMDs was particularly high among persons working in Tokyo. Therefore, these results indicate the need to investigate working conditions.

3. Questionnaire analysis of "clinical questions" for treating TMDs collected from dental care providers: A preliminary investigation of participants in the 20th annual meeting of the Japanese Society for the Temporomandibular Joint

The committee drafting guidelines for the primary care of TMDs of the Japanese Society for the Temporomandibular Joint performed a questionnaire survey of participants of the 20th annual meeting held on July 14 and 15, 2007. The purpose of the survey was to consider strategies for collecting clinical questions from health care providers. The questionnaire included the position in the society and social stage, the length of time treating TMDs, the format for clinical questions, and opinions regarding guidelines. Efforts to publicize the next investigation were considered necessary because we were able to collect completed surveys from only 61 respondents. Of these respondents, 54 (89%) were society members and 24 (39%) were authorized specialists. There were 31 dentists (51%) who had treated TMDs for more than 11 years. Of the symptoms described in the clinical question format, pain was the most frequent and was followed by joint noise and limited mouth opening. Splint therapy was the most common treatment and was followed by mouth-opening exercises and a pharmacotherapy. For several answers the responders seemed unable to recognize the format style for clinical questions. These findings suggest that more-detailed methods should be considered for collecting clinical questions.

4. Questionnaire analysis of general practice dentistry for the systematic understanding of clinical questions for clinical guidelines for TMDs

When drafting clinical guidelines, we should adopt the acronym "PICO": patient (or disease), intervention (a drug or test), comparison (another drug, placebo or test), and outcome of clinical questions.

Purpose: We performed a questionnaire survey of dentists in general practice to collate clinical questions for the treatment of TMDs.

Subjects and Methods: The Japan Dental Association (JDA) collected questionnaires, and we analyzed the secondary data, which eliminated personal information. All subjects were general members of the JDA or were members or nonmembers of the JDA working in participating clinics. In principle, 10% of the general members were extracted from every age group. To unify terms, one author compiled a list of similar terms using the text-mining method.
Results: We sent questionnaires to 5,999 dentists and received responses from 1,412 (response rate, 23.8%). Inadequate and incomplete clinical questions (353) were excluded from the total analysis set of 4,423, leaving an effective analysis set of 4,070. The main therapies (more than 5%) chosen for main symptoms (more than 3%) were 32 kinds of clinical questions.
Conclusion: These data and/or combination should be considered when drafting clinical guidelines for TMD.

*Morphological and histological studies of the temporomandibular joint*

1. Absence of an articular disk in the Tasmanian devil temporomandibular joint

Background: The articular disk of the temporomandibular joint is a constant structure in the Mammalia. According to Parson's report in 1900, however, the articular disk is absent in 4 mammals: the armadillo, 2 kinds of monotreme (the echidna and the platypus), and the Tasmanian devil. Since 1900, however, no research has been done to confirm this observation. The aim of this study was to determine by means of anatomical and histological examination whether the Tasmanian devil has an articular disk in its temporomandibular joint.
Methods: Fresh corpses of 8 Tasmanian devils were obtained from the School of Zoology, University of Tasmania. They were dissected, and the structure of the temporomandibular joint was carefully observed anatomically. Then, the temporomandibular joint was removed, immersed in 10% buffered formaldehyde solution, decalcified in 10% ethylenediaminetetraacetic acid solution, and embedded in paraffin. Serial sagittal sections were cut and stained with hematoxylin and eosin for histological examination.
Results: In all cases, gross observation and dissection revealed the absence of an articular disk. Histological examination showed that the surface layers of both the condyle and the glenoid fossa consisted of fibrous tissue thicker than that in other mammals. A synovial membrane-like structure was observed in the anterior and posterior parts of the fibrous structure of the condyle.
Conclusion: We confirmed the absence of an articular disk in the Tasmanian devil's temporomandibular joint. Furthermore, thick fibrous layers on the surfaces of both the condyle and the glenoid fossa might play a role as a buffer against hard jaw movement instead of articular disk.

2. Observation of the condyle using micro-computed tomography in the Tasmanian devil temporomandibular joint

Purpose: The aim of this study was to examine the structures of cancellous and cortical bone of the Tasmanian devil's condyle.
Methods: Fresh carcasses of 6 Tasmanian devils (5 male and 1 female; body weight: 4.3 to 10.4 kg) were obtained from the School of Zoology, University of Tasmania. One

of these dry skulls was obtained and used for micro-computed tomography (CT) examination. The condyle on the left side of the dry skull was examined with micro-CT (HMX-225 Actis 4, Tesco, Tokyo, Japan). Imaging was performed with a tube voltage of 140 kV, a tube current of 120 $\mu$A, a magnification of 6.0, and a slice width of 50 $\mu$m. Three-dimensional images were created with the volume-rendering method using Vgstudio (Nihon Visual Science Inc., Tokyo Japan). The findings were compared with those of a beagle.
Results and Conclusion: Sagittal and coronal micro-CT scans revealed dense and fine cancellous bone and thinner covering cortical bone in the condyle of the Tasmanian devil as compared with those of the beagle. These findings might be due to rapid turnover/renewal of bone as a result of powerful mastication and heavy loading on the condyle.

*Basic studies of oral mucosal keratinocytes*

1. Salivary trefoil factor 3 enhances migration of oral keratinocytes

Purpose: Trefoil factor (TFF) 3 is a member of the mammalian TFF family. TFFs are secreted onto mucosal surfaces of the entire body and exert different effects according to the tissue location. TFFs may enhance mucosal healing by modulating motogenic activity, inhibiting apoptosis, and promoting angiogenesis. TFF3 is secreted from the submandibular gland and is present in whole saliva. The aim of this study was to assess the migratory and proliferative effects of TFF3 on primary oral human keratinocytes and oral cancer cell lines.
Results: The addition of TFF3 increased the migration of both normal oral keratinocytes and the cancer cell line D12, as evaluated with a 2-dimensional scratch assay. In contrast, no increase in proliferation or energy metabolism was observed after stimulation with TFF3. The TFF3-enhanced migration was found to be driven partly by the extracellular signal—related kinase pathway, as shown by addition of the mitogen-activated protein kinase inhibitor PD 98059. Conclusion: All previous functional studies of trefoil peptides have been based on cells from monolayered epithelium, such as the intestinal mucosa; this is the first report to show that normal and cancerous keratinocytes from stratified epithelium respond to TFF stimuli. These findings suggest that salivary TFF3 contributes to oral wound healing.

2. Nerve growth factor and its receptors TrkA and p75NTR in the epithelium of oral lichen

Background: Nerve growth factor (NGF) can, through its receptors TrkA and p75NTR, convey signals for cell survival, differentiation, and death. The aim of this study was to examine whether NGF plays a role in the pathology of oral lichen.
Methods: Sections of biopsies from patients with erythematous oral lichen and from volunteers with normal oral mucosa were immunostained with antibodies against NGF, proNGF, TrkA, phosphorylated Trk, p75NTR, and phosphorylated Akt, and the expression of RNA coding for proNGF/NGF was investigated with *in situ* hybridization.
Results: Both in erythematous oral lichen and normal oral mucosa, cytoplasmic staining for NGF was seen in granular and upper spinous cell layers of the epithelium, whereas

proNGF staining was seen in all epithelial cell layers. In situ hybridization showed that the proNGF protein was produced in the same cell layers. In oral lichen, strong cytoplasmic staining for TrkA and phosphorylated Trk was observed in all epithelial cell layers, but staining was weak in normal oral mucosa. Basal keratinocytes in oral lichen showed no or only weak cytoplasmic staining for p75NTR, but in normal oral mucosa there was clear cell-membrane staining. In oral lichen, strong cytoplasmic and intermittent nuclear staining for phosphorylated Akt was observed in spinous, granular, and superficial layers, whereas basal and parabasal keratinocytes showed no staining. This staining was weak or absent in the entire epithelium of normal oral mucosa.
Conclusion: TrkA upregulation and activation in oral lichen is a pathway that can activate phosphorylated Akt and thereby rescue epithelial cells from untimely cell death.

**Publications**

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# Institute of DNA Medicine
# Department of Gene Therapy

Toya Ohashi, *Professor and Director* Hiroshi Kobayashi, *Assistant Professor*

## General Summary

Our purpose is to develop therapeutic methods, including gene and cell therapy, and we performed various studies and investigations this year. Below, we will describe the progress in each of our projects.

## Research Activities

*Genetic disease*

1. Lysosomal storage disease: The main achievements in our laboratory this year are as follows

1) Development of gene therapy for lysosomal storage diseases: We generated recombinant lentiviral vectors expressing the enzymes missing in Pompe disease, Krabbe disease, and mucopolysaccharidosis type VII (MPS VII), and administered these vectors to newborn model mice. For Pompe disease, enzyme expression and a reduction in glycogen storage were observed for 4 months in the heart. Moreover, there was no immunological response against enzymes or viral vectors and no liver damage. In Krabbe disease, we detected increased body weight but no effect on life span. In MPS VII, effects on body weight and life span were observed.

2) Screening for Pompe disease among patients with muscular dystrophy: We screened for Pompe disease with dried blood spots among patients in whom muscular dystrophy was diagnosed. This year, enzyme replacement therapy was started in a patient who was found to have Pompe disease.

3) Investigation of immune system in enzyme replacement for lysosomal storage diseases: In Fabry disease, we measured antibody titers and found that the level of antibody titer influenced alpha galctosidase A enzyme activities *in vivo*.

4) Establishment of induced pluripotent stem cells from patients with lysosomal storage diseases: Last year, we successfully established various induced pluripotent stem cells from mouse models of lysosomal storage diseases, such as Pompe disease, Fabry disease, and MPS VII. This year, we successfully caused the stem cells to differentiate into cardiac cells.

5) Functional analysis of lysosomal storage diseases: Last year, we established vascular endothelial cells from patients with Fabry disease. This year, we used the monocyte chemoattractant protein (MCP) 1 knockout mouse and the twitcher mouse, which are models of Krabbe disease, to investigate the role of MCP1 in Krabbe disease. MCP1 is a chemokine and an important factor in inflammation.

2. Diabetes mellitus: Gene- and cell-based therapies for diabetes mellitus

To recover pancreatic beta cell mass and physiological control of glucose metabolism in

diabetes mellitus, *in vivo* gene transfer of cyclin-dependent kinase 4, a regulatory factor of the cell cycle, was performed in an animal with diabetes. Sixteen weeks after treatment, beta cell mass had increased 2.5-fold compared with that in a mock-transfer animal. Increased replication of the terminally differentiated beta cells is implicated as the mechanism of the beta cell increase.

3. Hepatocellular carcinoma and pancreatic carcinoma

1) Gene therapy for liver tumors

In our previous study, we developed methods of gene therapy for hepatocellular carcinoma (HCC) and for metastasis to the liver, which is an important prognostic factor for gastrointestinal cancers, and demonstrated antitumor effects against HCC and cancers metastatic to the liver with an adenovirus-mediated transfer of CD40 ligand that stimulated antitumor immunity. In this study, we explored a gene-therapeutic approach to treat these cancers with a naked plasmid DNA of CD40 ligand.

2) Examination of antitumor effects of protease inhibitors against pancreatic carcinoma

In a previous study we investigated antitumor effects of the synthetic serine protease inhibitor nafamostat mesilate, which inhibited nuclear factor NF $\kappa$B, which is involved in the proliferation, metastasis, and chemoresistance of many tumors, and confirmed the antitumor effects of nafamostat mesilate, alone and in combination with gemcitabine, in a human pancreatic carcinoma cell line. In this study, we examined the antitumor effects of the combination of nafamostat mesilate and paclitaxel in mice with metastatic pancreatic carcinoma.

4. Gynecologic oncology

1) Integrated copy number and expression analysis of chemoresistant ovarian carcinomas

Women with serous ovarian cancer are often intrinsically refractory to platinum-taxol-based treatments or become resistant on relapse. Because accurately predicting the response to chemotherapy remains possible, we sought to identify somatic DNA copy number variation (CNV) associated with primary resistance in advanced-stage disease. Genome-wide frequency and the level of CNV in 118 ovarian tumors were measured with single nucleotide polymorphism microarrays. A well-defined subset of 85 advanced-stage serous tumors was then used to relate CNV to primary resistance to treatment. The discovery-based approach was complemented by quantitative polymerase chain reaction analysis of copy number of 12 candidate genes previously reported to be associated with clinical outcome in ovarian cancer. Likely CNV targets and tumor molecular subtypes were further characterized by gene expression profiling. Amplification of 19q12, containing cyclin E (CCNE1) and 20q11.22-q13.12, mapping immediately adjacent to the steroid receptor co-activator NCOA3, were significantly associated with a poor response to primary treatment. From previously reported associations of copy number with outcome, only the amplification status of CCNE1 was validated as a marker for primary chemoresistance. Chemoresistant tumors with high CCNE1 copy number and protein expression were predictably associated with increased cellular proliferation, as were a subset of treatment-responsive patients, suggesting a cell-cycle-independent role for CCNE1 in modulating chemoresponse. Patients with poor clinical outcomes and without CCNE1 amplification over-expressed genes involved in

extracellular matrix deposition. Our findings identify 2 distinct mechanisms of primary treatment failure in serous ovarian cancer, involving CCNE1 amplification and enhanced extracellular matrix deposition.

2) Mesenchymal-to-epithelial transition during inclusion cyst formation from human ovarian surface epithelium

Most surface epithelial-stromal tumors of the ovary are thought to arise from epithelial inclusion cysts. Thus, these cysts are precursor lesions of ovarian carcinoma. On the basis of this hypothesis, we aimed to characterize the human ovarian surface epithelium in which the mesenchymal-to-epithelial transition occurs in the process of inclusion cyst formation. We used specimens from 9 patients with endometrial cancer who had undergone hysterectomy and bilateral salpingo-oophorectomy. Immuohistochemical studies were performed of 4 normal fallopian tubes and 10 normal ovaries containing 92 inclusion cysts to examine the expression of antigen markers, including calretinin, podoplanin, D2-40, thrombomodulin, human bone marrow endothelial (HBME)-1, vimentin, epithelial membrane antigen (EMA), WT1, carbohydrate antigen (CA) 125, MOC31, tumor-associated glycoprotein (TAG) 72, Ber-EP4, and E-cadherin. We found that positive staining rates for mesothelial markers in normal ovarian surface epithelium were 100% (10 of 10) with calretinin, 80% (8 of 10) with podoplanin, 80% (8 of 10) with D2-40, 70% (7 of 10) with thrombomodulin, 100% (10 of 10) with HBME-1, and 100% (10 of 10) with vimentin; that positive staining rates for epithelial markers in tubal epithelium were 100% (4 of 4) with HBME-1, 100% (4 of 4) with vimentin, 100% (4 of 4) with EMA, 75% (3 of 4) with TAG-72, and 100% (4 of 4) with Ber-EP4; and that positive staining rates for both markers in inclusion cysts were 51.1% (47 of 92) with HBME-1, 44.6% (41 of 92) with vimentin, 65.2% (60 of 92) with TAG-72, and 88.0% (81 of 92) with Ber-EP4. Ovarian surface epithelium has both mesencyhmal and epithelial characteristics. In contrast, inclusion cyst gains more epithelial characteristics with the loss of mesencyhmal characteristics. These findings support a mesenchymal-to-epithelial transition during inclusion cyst formation from ovarian surface epithelium.

3) Fetomaternal medicine

Establishment of an immortalized human extravillous trophoblast cell line by retroviral infection of E6/E7/human telomerase reverse transcriptase

Investigation into the function of human trophoblasts has been restricted by a lack of suitable cell models. We aimed to obtain long-lived human normal trophoblast cell lines that would serve as ideal *in vitro* cell models. Primary human trophoblast cells were derived from the placenta of a woman who had undergone elective abortion during the 7th week of gestation. The cells were immortalized by infection with retroviral expression vectors containing type 16 human papillomavirus E6 and E7 in combination with human telomerase reverse transcriptase (hTERT). Characterization of the cell line was performed. Immunocytochemical staining for human chorionic gonadotrophin chain $\beta$, cytokeratin 7, human leukocyte antigen G, and CD9 indicated an extravillous trophoblastic phenotype. Transwell insert invasion assay showed the invasiveness of this cell line, and gelatin zymography showed secretion of matrix metalloproteinases 2 and 9. Karyotype analysis showed almost normal chromosomal number with small deviations ranging from 46 to 48, and a nude mouse assay showed no tumorigenecity.

This newly immortalized cell line, HChEpC1b, will provide a useful model for the study of extravillous trophoblast function.

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# Institute of DNA Medicine
# Department of Oncology

Mikio Zeniya, *Professor*
Junko Horiguchi-Yamada, *Associate Professor*
Sadamu Homma, *Associate Professor*
Shigeo Koido, *Assistant Professor*

## General Summary

Our research focuses on tumor immunology and leukemia cell biology. Experiments were performed to enhance the beneficial effects of immunotherapy, to clarify differentiation mechanisms, and to investigate the pathophysiology of hepatitis induced by autoimmune mechanisms. Two clinical studies of immunotherapy are in progress.

## Research Activities

*Combined treatment with dendritic cells and 5-fluorouracil elicits augmented natural killer cell-mediated antitumor activity via the tumor necrosis factor-alpha pathway*
Inoculation of mice with dendritic cells (DCs) increased the number of natural killer (NK) cells in the spleen and up-regulated tumor necrosis factor (TNF)-alpha expression on NK cells. Pretreatment with 5-fluorouracil (5-FU) enhanced expression of procaspase 8 in mouse colon cancer cells and induced apoptosis by TNF-$\alpha$ through the caspase 8 pathway. These results indicate that combined therapy with a DC vaccine and 5-FU is a promising strategy for cancer treatment.

*Mechanisms of synergistic effect of WT1 peptide vaccine and gemcitabine against pancreatic cancer*
Treatment with gemcitabine increased expression of WT1 (Wilms' tumor gene) messenger RNA in pancreatic cancer cells. WT1-specific $CD8^+$ cytotoxic T lymphocytes killed the gemcitabine-treated pancreatic cancer cells more efficiently. These experiments indicate that cytotoxic effects of WT1-specific cytotoxic T lymphocytes induced by a WT1 peptide vaccine would be enhanced by gemcitabine treatment in a patient with pancreatic cancer.

*The identification of novel tumor-associated antigens from a mouse model of familial adenomatous polyposis*
Immunization of mice with DCs fused with tumor cells established from the intestinal tumors of a familial adenomatous polyposis mouse induced antibody production. The antigen recognized by this antibody showed homology with melanoma inhibitory activity 3 and was present in both Paneth cell-like tumor cells and normal Paneth cells. This novel antigen might function as a modulator of commensal microbiota.

*Phase I clinical trial of combination therapy with WT1 peptide vaccine and gemcitabine against advanced pancreatic cancer*

The modified 9-mer WT1 peptide (235–243AA, CYTWNQMNL for HLA-A*2402) has been used as a cancer vaccine. A phase I clinical trial of combination therapy with WT1 peptide vaccine and gemcitabine against advanced pancreatic cancer was performed in collaboration with Osaka University. In 2008, 2 patients were enrolled in the study, and no severe adverse effects were observed. One patient showed marked tumor regression (final tumor regression rate, 82%) with disappearance of symptoms.

*Clinical immunotherapy for brain tumor*

The combination of fusion-cell therapy with the chemotherapy has been on-going. Fusion cells have been produced in the Good Manufacturing Practice cell-processing system.

*Adhesion-induced differentiation toward megakaryocytes*

Adhesion to fibronectin and collagen induces megakaryocytic differentiation through activation of the FLI-1 gene, a member of the ets-family of transcription factors, in human leukemia JAS-R cells. We elucidated the mechanisms of the activation of this gene and found that FLI-1 functions in an autoaugmentation fashion once it is activated by adhesion.

*Accumulation of functional regulatory T cells in actively inflamed liver in mouse DC-based autoimmune hepatic inflammation*

Regulatory T cells accumulated in the liver through interaction of chemokine (C-X-C motif) receptor 3 and chemokine (C-X-C motif) ligand 9 and expanded locally by stimulation of transforming growth factor-beta and interleukin-2 in autoimmune hepatic inflammation. An increase in the number of functional regulatory T cells might be a protective reaction and might play a role in the severity and persistence of autoimmune hepatic inflammation.

## Publications

***Homma S, Koido S, Sagawa Y, Suzuki H, Komita H, Nagasaki E, Takahara A, Horiguchi-Yamada J, Tajiri H, Zeldin D, Obata T.*** Antigenic stimulation with cytochrome P450 2J expression in mouse hepatocullular carcinoma cells regulates host antitumor immunity. *Clin Exp Immunol* 2009; **156:** 344–52.

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***Horiguchi-Yamada J, Suzuki H, Takeda N, Yamada H.*** Sequential alterations in cell cycle and survival after X-ray Irradiation in p53-deficient leukemia cells. *Cytomet Res* 2009; **19:** 53–60.

***Homma S, Koido S, Obata T.*** Proteomics-based exploration of T-cell epitopes from tumor associated antigens for generation of new cancer vaccine (in Japanese). *Biotherapy* 2008; **22:** 385–8.

# Institute of DNA Medicine
# Department of Molecular Genetics

Hisashi Yamada, *Director and Professor*
Masaharu Akiyama, *Assistant Professor*
Takeshi Kawano, *Assistant Professor*
Takaaki Hayashi, *Assistant Professor*

## General Summary

The research goal of our department is to develop prophylactic and therapeutic strategies based on disease-specific etiology. Major target diseases are malignant tumors, including hematological and pediatric malignancies. Molecular pharmacological studies of anticancer agents are another area of other research. In particular, we are investigating histone deacetylase inhibitors (HDACIs). We are also investigating the molecular etiology of spinal muscular atrophy (SMA) and retinal diseases.

## Research Activities

*Exploring leukemogenesis*

Several clinical trials for acute leukemias have been performed in the last 2 decades, but except for the treatment of acute promyelocytic leukemia, no protocols have been proven to have a therapeutic advantage over the basic combination chemotherapy proposed in the early 1990s. On the other hand, basic research on leukemia has made extraordinary progress. Leukemic stem cells and the structure of hematopoietic stem cell niche were discovered, and these discoveries have shown why leukemias are resistant to intensive chemotherapies. Megakaryocytic leukemia has an extremely poor prognosis in adult patients. We have recently established a cell line derived from a patient with this disease. This cell line, named JAS-R, show megakaryocyte and erythroblast phenotypes and is ideal for studying the lineage shift between megakaryocytes and erythroblasts. The lineage shift of JAS-R cells is governed by the adherence to culture dishes. Several transcription factors are involved in the lineage determination. Among them, Friend leukemia virus integration 1 (FLI1), a member of the E twenty-six (ETS) gene family, has been shown to play an important role in megakaryocytic differentiation. Therefore, we are now studying the FLI1 induction mechanism along with the cell-adhesion process.

*Molecular pharmacology of anticancer agents*

Based on the understanding of cancer genomics, many new agents, called molecularly targeted drugs, have been developed. However, the single administration of most new drugs has failed to show a sufficient effect in patients. Thus, combination protocols have been developed. Among the molecularly targeted drugs, HDACIs and their pharmacology have been our focus. Acetylation and deacetylation of histones regulate gene transcription through chromatin remodeling. Transcription genes related to cell survival and death are affected by HDACI treatment. We are studying whether

HDACIs can augment cell cytotoxicity by being administered with conventional anticancer drugs and ionizing radiation. In fact, one HDACI, valproic acid, has shown a synergistic effect with a DNA-topoisomerase I inhibitor. This seems to be a result of induction failure of the antiapoptotic protein B-cell lymphoma 2 extra large (Bcl-xL) by a DNA-topoisomerase I inhibitor. We are also studying the effect of the combination of HDACIs and ionizing radiation against retinoblastoma cells. The dose of radiation to induce apoptosis of cells was reduced to 20% by simultaneous treatment with an HDACI. Unexpectedly, this augmentation was due to the stabilization of a p53-tumor suppressor protein. We are now studying the molecular mechanisms of this stabilization.

*Pathogenesis of spinal muscular atrophy*

SMA is a degenerative disorder that leads to muscular atrophy. The mutation of the SMA1 gene is responsible for the onset of the disease. However, unlike other mammals, human beings have SMA2, a gene of the same family as SMA1. We are studying why intact SMA2 cannot produce a sufficient amount of SMA protein in patients with SMA.

**Publications**

***Akiyama M, Yamada O[1], Agawa M, Yuza Y, Yanagisawa T, Eto Y, Yamada H ([1]Tokyo Women's Med Coll).*** Effects of predonisolone on specifically expressed genes in pediatric acute B-lymphoblastic leukemia. *J Pediatric Hematol Oncol* 2008; **30:** 313–6.

***Horiguchi-Yamada J, Suzuki H, Takeda N, Yamada H.*** Sequential alterations in cell cycle and survival after X-ray irradiation in p53-deficient leukemia cells. *Cytomet Res* 2009; **19:** 53–60.

***Agata N, Ahmad R[1], Kawano T, Raina D[1], Kharbanda S[1], Kufe D[1] ([1]Harvard Univ).*** MUC1 oncoprotein blocks death receptor-mediated apoptosis by inhibiting recruitment of caspase-8. *Cancer Res* 2008; **68:** 6136–44.

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# Institute of DNA Medicine
# Department of Molecular Immunology

Saburo Saito, *Associate Professor and Director*
Daitaro Kurosaka, *Associate Professor*
Yuji Ohno, *Assistant Professor*

## General Summary

Our research interests have focused on the analysis of the basic immune system to protect us from diseases and of immune disorders, such as hypersensitivity diseases and autoimmune diseases.

## Research Activities

*The role of CRTH2 in Th1 and Th2 inflammatory reactions*

Many reports support the concept that chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) mediates the proinflammatory effects of prostaglandin (PG) $D_2$ in Th2-related inflammatory reactions. However, little is known about the Th1-related inflammatory reactions. The aim of this study was to investigate the role of CRTH2 in various inflammatory conditions. To induce Th2-related inflammatory reactions, BALB/c mice were infected with *Nocardia brasiliensis,* and serum IgE levels, numbers of eosinophils, and the antigen-specific Th2-cytokine production of spleen cells were measured 2 weeks after infection. To induce Th1-related inflammatory reactions, BLAB/c mice were given subcutaneous injections of complete Freund adjuvant, and interferon $\gamma$ production was measured for 72 hours in a culture of draining lymph node cells stimulated with purified protein derivative. To analyze the role of CRTH2, ramatroban, a selective CRTH2 antagonist, was orally administered to some mice to inhibit CRTH2 signaling *in vivo.* In addition, CRTH2-knockout mice were also used for further analysis of the role of CRTH2. In mice infected with *N. brasiliensis,* ramatroban administration inhibited IgE production, eosinophilia, and Th2 cytokine production. In contrast, in mice treated with complete Freund adjuvant, ramatroban administration enhanced interferon $\gamma$ production. Similar results were obtained in CRTH2-knockout mice. These results indicate that CRTH2 mediates the proinflammatory effects of $PGD_2$ in Th2 inflammatory reactions and mediates the anti-inflammatory effects of $PGD_2$ in Th1 inflammatory reactions.

*Enhancing activity of N-glycosylation for constitutive proteins secretions in nonpolarized cells*

Several fusion proteins of mouse (m) interleukins (ILs) and the enhanced green fluorescent protein were expressed in fibroblasts and epithelial cells. Among these proteins, the mIL-31 derivative was the most efficiently secreted into the medium in a N-glycosylation—dependent manner. Analysis of deletion mutants revealed that the minimal structure for constitutive secretion consisted of a signal peptide and N-

glycosylation. Introduction of the signal sequence from mIL-31 to human p53 protein failed to lead to the secretion of the products, but further addition of the N-glycosylation site resulted in constitutive secretion of biologically active p53 protein into the medium in the N-glycosylated form. This report has shown the importance of N-glycosylation for constitutive protein secretion, especially in nonpolarized cells.

*Alteration of inflammatory cytokine production in the injured central nervous system of tenascin-deficient mice*

Although tenascin-C (TN) is highly up-regulated during the proliferation of reactive astrocytes, little is known about the function of TN at injury sites in the central nervous system. We investigated the function of TN-expressing astrocytes in the injured brain by analyzing TN-deficient mice with stab-wound injuries of the cerebral cortex. Expression of glial fibrillary acid protein was down-regulated earlier after injury in TN-deficient mice than in wild-type (WT) mice. To evaluate immune responses in the injured central nervous system in the absence of TN, inflammatory cytokine production was examined after unilateral stab injuries of the cerebral cortex in TN-deficient and WT mice. The expression of IL-1 beta, tumor necrosis factor-alpha, and IL-6 was higher in TN-deficient mice, whereas levels of IL-4 and granulocyte colony-stimulating factor were lower in TN-deficient mice than in WT mice. Our findings suggest that TN helps to regulate the production of inflammatory cytokines in the injured brain.

## Publications

***Akiyama N, Ohno Y, Fukuda T, Manome Y, Saito S.*** Enhancing activity of *N*-glycosylation for constitutive proteins secretions in non-polarized cells. *Biochem Biophys Res Commun* 2009; **381:** 612–8.

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# Institute of DNA Medicine
# Department of Molecular Cell Biology

Yoshinobu Manome, *Professor and Director*
Hiroyuki Sasaki, *Associate Professor*
Toru Obata, *Associate Professor*
Michiko Watanabe, *Assistant Professor*

## General Summary

Our research goals include molecular analysis and visualization of cellular events under both physiological and pathological conditions. To achieve these goals, we have used morphological and biochemical approaches. Our department has two sections: biochemistry and fine morphology. Through the activities of both sections, we are exploring medical life sciences.

## Research Activities

*Development of sonodynamic therapy and diagnostics for malignant glioma*

Ultrasound has been widely used as a diagnostic tool. It is handy, convenient, and inexpensive. It is also safe, because no ionizing radiation or other harmful energies are emitted. Thus, many clinicians and medical technologists use ultrasound. Recently, therapeutic ultrasound irradiation, or insonation, has been developed. Insonation is a potentially useful cancer treatment. One application is sonodynamic therapy. When sonodynamic agents are enhanced with ultrasound, insonation has cytocidal effects on nearby malignant tissues. With this method, we are developing a therapeutic strategy for malignant brain tumors. A microbubble agent, Levovist, is used as an ultrasound enhancer, and both therapy and diagnosis can be simultaneously achieved.

*Three-dimensional cell culture of malignant glioma cells*

Cell culture is a basic tool for understanding the characteristics of tissues and organs in the human body. The procedure is also essential for the development of diagnostics and therapeutics for human disease. However, vital cellular functions that are present in tissues or organs are missed by ordinary flask-based or culture dish-based cell cultures. From this point of view, we have established a culture method that mimics the human intracranial environment. This year, we compared 4 different malignant glioma cell lines. A bioadaptable and biodegradable gelatin was used as a scaffold upon which cells were cultivated. Some morphologic features observed in 3-dimensional (3D) culture could not be observed in conventional cell culture. When the 4 glioma lines were compared, each cell line demonstrated distinct characteristics. For example, 1 cell line conglomerated and formed balloon-like structure, and cells of another line dispersed and grew separately immediately after cell division. These characteristics were unpredictable and could be revealed only with the current culture method. We conclude that this culture method is useful for evaluating characteristics of individual glioma cell lines in the human body.

*Functional analysis of tight junctions*

Tight junctions (TJs) in the epithelia and endothelia restrict the paracellular flux of water and solutes. In the epidermis, the significance of the TJ is largely unknown because of the structural complexity of the epidermis. To understand TJ functions in the epidermis, a specific method for TJ disruption would be useful. Sodium caprate is a well-known absorption enhancer that causes dilatation of the TJ and increases paracellular permeability in the intestine. We investigated the effects of sodium caprate on 3D cultures of human skin to help understand TJ functions in the epidermis. After treatment with sodium caprate, transepidermal resistance decreased, indicating paracellular barrier disruption. Treatment with sodium caprate decreased claudin-1 and occludin expression and fragmented their localization in 3D skin cells. Cell polarity was disrupted in 3D skin as well. These results suggest that sodium caprate induces TJ disruption in 3D cultures of human skin and can be applied to further studies of epidermal TJ function.

*Photoluminescent silicon quantum dots*

In nanotechnology research, we assessed biochemical applications of photoluminescent silicon (Si) quantum dots (QDs). Si-QDs have been used as biological labels for imaging living cells at nontoxic concentrations. We have shown that Si-QDs have no toxicity against living cells at a concentration of 112 $\mu$g/mL and that Si-QDs are less toxic than current cadmium-selenium (CdSe)-QDs at high concentrations both in modified methylthiotetrazol assays and with lactate dehydrogenase assays. We found that under ultraviolet light CdSe-QDs released cadmium and were more toxic than nonirradiated CdSe-QDs or Si-QDs. In addition, we found that the toxicity mechanisms of Si-QDs at high concentrations were related to radical production. These results will be useful for the future application of Si-QDs in biology and medicine.

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# Institute of DNA Medicine
# Project Laboratory for Kidney Regeneration

Takashi Yokoo, *Director*

## General Summary

Kidney regeneration is gaining considerable attention as the ultimate treatment strategy for renal failure, thus replacing kidney dialysis. However, the kidney is believed to be the most difficult organ to regenerate because it is anatomically complicated and because each cell in the kidney must be precisely placed to achieve renal function. Therefore, it is difficult to imagine such a complicated organ being created from pluripotent stem cells through genetic or chemical manipulation. However, in every individual this sophisticated structure is present at birth in the proper position due to perfect programming of the developmental kidney during embryogenesis. Therefore, if we can unravel this entire process and follow it, kidneys could be regenerated even after birth. We are investigating the potential for reconstructing an organized and functional kidney structure, using the developing xeno-embryo as an organ factory.

## Research Activities

*A thermoreversible polymer mediates controlled release of glial-cell—derived neurotrophic factor to enhance kidney regeneration*

We have previously reported that human mesenchymal stem cells (hMSCs) cultivated in growing embryos differentiated in an appropriate developmental milieu, thereby facilitating the development of a functional renal unit. However, this approach required transfection with an adenovirus that expressed glial-cell—derived neurotrophic factor (GDNF) to enhance the development of hMSC-derived renal tissue, and safety issues restrict the clinical use of such viral vectors. To circumvent this problem, we tested an artificial polymer as a means to diffuse GDNF. This GDNF polymer, which exists in liquid form at 4°C but becomes a hydrogel upon heating to 37°C, was used as a thermoreversible switch, allowing the injection of hMSCs at low viscosity using a mouth pipette, with subsequent slow diffusion of GDNF as it solidified. The polymer, which was dissolved in a solution of GDNF at 4°C and then maintained at 37°C, acted as a diffuser of GDNF for more than 48 hours. *LacZ*-transfected hMSCs and the GDNF polymer (at 4°C) were placed in nephrogenic sites of growing rat embryos maintained at 37°C. Forty-eight hours later, the resultant kidney anlagen were dissected out and allowed to continue developing for 6 days *in vitro*. Whole-organ X-Gal staining and fluorescence-activated cell sorter analysis showed that the number of hMSC-derived cells was significantly greater in developed anlagen that have been generated from hMSCs plus GDNF polymer than in anlagen generated from hMSCs plus GDNF-containing medium and was comparable to those from adenovirus-transfected hMSCs. These findings suggest that the GDNF polymer can be used as a diffuser of GDNF for kidney organogenesis.

## Publications

***Matsumoto K, Yokoo T, Fukui A, Kawamura T, Ohashi T, Kobayashi E, Hosoya T.*** Physiological development of early metanephroi in paraaortic area as a source for xenotransplantation. (submitted)

***Gheisari Y, Yokoo T, Matsumoto K, Fukui A, Sugimoto N, Ohashi T, Kawamura T, Hosoya T, Kobayashi E.*** A thermoreversible polymer mediates controlled release of GDNF to enhance kidney regeneration. Artif Organs (in press)

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# Department of Neuroscience
# Division of Neuropathology

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

*Tumor markers in pineal parenchymal cell tumor*

Pineal parenchymal tumor (PPT) cells usually show immunoreactivity for synaptophysin, neuron-specific enolase, neurofilament protein, class III $\beta$-tubulin, tau protein, protein gene product 3.5, chromogranin, serotonin, retinal S-antigen, and rhodopsin. These markers, however, are not specific for PPTs. Melatonin is produced and secreted mainly by the pineal parenchymal cells.

Hydroxyindol-O-methyltransferase (HIOMT) catalyzes the final reaction in melatonin biosynthesis. We hypothesized that HIOMT could serve as a marker of PPTs. We investigated HIOMT localization in humans and HIOMT expression in PPTs, primitive neuroectodermal tumors, and medulloblastomas. In human tissue, HIOMT was expressed in retinal cells, pineal parenchymal cells, neurons of the Edinger-Westphal nucleus, microglia, macrophages, thyroid follicular epithelium, principal and oxyphil cells of the parathyroid gland, adrenal cortical cells, hepatic parenchymal cells, renal tubule epithelial cells, and enteroendocrine cells of stomach and duodenum. HIOMT was expressed in all PPTs studied. The ratio of HIOMT-immunoreactive cells successively decreased in the following tumors: pineocytoma, pineocytomatous areas of PPTs with intermediate differentiation (PPTIDs), pineoblastomatous areas of PPTIDs, and pineoblastomas. In 1 of 3 primitive neuroectodermal tumors and 4 of 8 medulloblastomas, a few HIOMT-immunoreactive cells were observed. Immunohistochemical analysis of HIOMT is useful for diagnosing PPTs, and HIOMT may be a prognostic factor in patients with PPTs.

*Expression array analysis of a spinocerebellar ataxia type 7 cell model*

Spinocerebellar ataxia type 7 (SCA7) is a polyglutamine disease caused by polyglutamine expansion within a causative protein, ataxin-7. SCA7 is characterized by specific degeneration of cerebellar, brainstem, and retinal neurons. Recent evidence suggests that ataxin-7 regulates transcription and that aberrant regulation of transcription is involved in the pathogenesis of SCA7, yet additional studies are be needed to clarify

the pathogenesis of SCA7. We developed a PC12 inducible cell line expressing mutated ataxin-7 (ataxin-7-Q100). In this cell line, expression of the mutated ataxin-7 is regulated by the presence of tetracycline in the culture medium. Expression array analysis of the cell line was performed to compare gene expression levels between the cells with and without induction of the mutated ataxin-7. Approximately 40,000 genes were analyzed: of these genes. 600 showed increased expression with induction of the mutated ataxin-7, and 300 genes showed decreased expression. Suppression of several retinal specific genes was identified, indicating that the fluctuating expression of these genes might be involved in the retinal pathology of SCA7.

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# Department of Neuroscience
# Laboratory of Neurophysiology

Fusao Kato, *Professor and Director*

## General Summary

The integration of functions throughout the entire body is realized mainly through intercommunication via the nervous system. To understand how the activities of the organs affect brain activity and, in turn, how the brain controls the activities of the organs to optimize these integrative functions, it is absolutely necessary to clarify the mechanisms underlying the dynamic cell-to-cell signaling in the central nervous system underlying various specific functions, such as autonomic regulation and pain sensation. We use approaches at the molecular, cellular, and network levels, including patch-clamp recording of synaptic currents and real-time imaging of the intracellular $Ca^{2+}$ concentration in living brain tissues from normal animals, animal models of various diseases, and animals with experimental manipulation of gene expression.

## Research Activities

*Central mechanisms of pain-related negative emotion*

Using a rat model of chronic neuropathic pain, we demonstrated that structural consolidation is involved in synaptic potentiation at excitatory synapses between afferent fibers arising from the nucleus parabrachialis and neurons in the central nucleus of the amygdala, a structure playing a principal role in the expression of emotional behavior.

*Glia-neuron interaction at central synapses*

1. Astrocyte network activation through ATP receptors

In the nucleus of the solitary tract, we have demonstrated that astrocytes form a horizontally organized network with specific process extension and gap junction-mediated connections. We demonstrated that activation of P2Y1 receptors increases intracellular Ca concentrations at these processes, suggesting that processes, rather than the soma, are the functional units for glia-neuron interaction.

2. The role of monocarboxylate transport in the synaptic function

To clarify the role played by the transfer of lactate from astrocytes to neurons in synaptic transmission, we analyzed the effect of a selective inhibitor of monocarboxylate transporters on synaptic transmission in neurons of the nucleus of the solitary tract and found that lactate transport is needed to maintain the postsynaptic responses mediated by AMPA receptors, both in the presence and absence of glucose supply.

3. RNA interference modulation of presynaptic ATP receptor properties

To clarify the roles played by specific molecules in transmitter release in brain synapses, we developed a novel method for *in-vivo* gene silencing with RNA interference against the genes coding presynaptic proteins. We succeeded in altering pharmacological

characteristics of the presynaptic P2X receptors underlying extracellular ATP-evoked transmitter release, following reduced expression of subunit mRNA and protein by injection of small interfering RNA into the nodose ganglion.

## Publications

***Takahashi Y, Ikeda R, Kato F.*** Distinct mechanisms of synaptic potentiation in the central amygdala in distinct pain models (in Japanese). *Pain Res* 2009; **24:** 137–46.

***Sumiyama, K, Tajiri, H, Kato F, Imura T, Ono K, Ikeda K, Imazu H, Gostout CJ.*** Pilot study for in vivo cellular imaging of the muscularis propria and *ex vivo*-molecular imaging of myenteric neurons. *Gastrointest Endosc* 2009; **69:** 1129–34.

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# Department of Genetic Disease Research (Lysosomal Storage Disease)

Yoshikatsu Eto, *Professor*
Toya Ohashi, *Professor*
Hiroyuki Ida, *Professor*
Hiroshi Kobayashi, *Assistant Professor*

## General Summary

The Donated Department of Genetic Disease Research (Lysosomal Storage Disease [LSD] Research Center) was established in April 2008. The main topic of research is the basic pathogenesis of genetic diseases, especially LSDs. In particular, the pathogenesis of central nervous system (CNS) involvement in LSDs is a difficult problem. To understand the pathophysiology of CNS events, we attempted to generate induced pluripotent stem (iPS) cells from mucopolysaccharidosis (MPS) VII mice and to differentiate them into neuronal cells. For Fabry disease and Pompe disease, we generated iPS cells from model mice and differentiated them into cardiac cells.

## Research Activities

1. Our aim is to establish novel treatment procedures, such as enzyme replacement therapy (ERT), chaperon therapy, new anti-oligonucleotide therapy (PTC), and cell therapy/gene therapy.

1) Hematopoetic stem cell transplantation

Hematopoetic stem cell transplantation was performed to treat Hurler disease, Hunter disease, Gaucher disease, Krabbe disease, and metachromatic leukodystrophy.

2) ERT

Six LSDs can now be treated with ERT.

3) Small molecules:

Substrate deprivation therapy and chaperon therapy was performed with butylnojirimycin (miglustat), Genz , and N-octyl-4-epi-beta-valienamine.

4) Gene Therapy

Gene therapy was performed with viral vectors (including adeno-associated viruses, adenoviruses, retroviruses, and lentiviruses), *ex vivo, in vivo* gene therapy.

5) Cell Therapy

Cell therapies involved neural stem cell therapy performed by means of intraventricular or intravenous injections, intranvenously administered mesenchymal stem cell therapy, fibroblast-derived iPS cells (Yamanaka, 2007), embryonic stem cells, and microglial cells. Among these treatments, we explored ERT and gene therapy in human and animal models, particularly in Pompe disease mice, using lentivirus vectors.

2. Establishing new iPS cells from various LSDs: We successfully derived iPS cells from the skin fibroblasts of twitcher, Fabry, and Sly mice. To derive iPS cells from tail-tip fibroblasts and mouse embryonic fibroblasts, 4 factors were inserted (hKlf4, hSox2, hc-Myc, and hOct), and Myc was deleted (Mao et al., 2008).

3. Evaluating the long-term efficacy of ERT for LSDs: Enzyme uptake in Gaucher disease occurs through a mannose receptor—mediated system. High mannose-6-phosphate—enriched enzymes are taken up at a high rate. Antibody formation will also inhibit the uptake of enzymes by cells. The relation to antibodies is the most important factor for ERT.
4. New treatments for CNS involvement in LSDs and other genetic diseases
Generally, ERT is not effective for treating neurological improvement, but we have performed intrathecal administration of enzymes into MPS II mice.
5. New screening procedures for LSD with dried blood spots and other techniques
We have attempted to develop procedures for screening for the development of Pompe disease with dried blood spots.

### Publications

***Ohashi T, Iizuka S, Ida H, Eto Y.*** Reduced alpha-Gal A enzyme activity in Fabry fibroblast cells and Fabry mice tissues induced by serum from antibody positive patients with Fabry disease. *Mol Genet Metab* 2008; **94:** 313–8.
***Hanyu K, Iida T, Shiba H, Ohashi T, Eto Y, Yanaga K.*** Immunogene therapy by adenovirus vector expressing CD40 ligand for metastatic liver cancer in rats. *Anticancer Res* 2008; **28:** 2785–9.
***Iida T, Shiba H, Misawa T, Ohashi T, Eto Y, Yanaga K.*** Adenovirus-mediated CD40L gene therapy induced both humoral and cellular immunity against rat model of hepatocellular carcinoma. *Cancer Sci* 2008; **99:** 2097–103.
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***Kitagawa T, Suzuki K, Ishige N, Ohashi T, Kobayashi M, Eto Y, Tanaka A, Odaka H, Owada M.*** Non-invasive high-risk screening for Fabry disease hemizygotes and heterozygotes. *Pediatr Nephrol* 2008; **23:** 1461–71.
***Miyata I, Yoshikawa H, Kurokawa N, Kanno K, Hayashi Y, Eto Y.*** A neonatal case of autosomal dominant hypoparathyroidism without mutation of the CASR gene. *Clin Pediatr Endocrinol* 2008; **17:** 17–22.
***Miyata I, Yoshikawa H, Ito C, Waki E, Sasaki O, Kumagaya E, Saito K, Ida H, Eto Y.*** Attempt to provide a team healthcare using an outpatient clinical path for growth hormone deficient short statue. *J Pediatr Pract (in press)*

### Reviews and Books

***Eto Y.*** Recent advances in clinical and molecular genetics. *J Pediatr Pract* 2009; **1:** 13–6.
***Eto Y.*** What will be required for future pediatric medical care. *J Pediatr Pract* 2008; **11:** 17–8.
***Eto Y.*** The guideline for diagnosis and treatment in Pompe disease and the difficulties in supporting patients and their famiry: Japanese journal of home health care for patients with intractable diseases (Nanbyo to Jutaku-kea). *Japan Planning Service Center* 2008; **1:** 48–50.
***Eto Y.*** Congenital (Inherited) metabolic disorders: inborn error of lipid metabolism. Respiration syndrome (version 2). *Shin-Ryoikibetsu Syokogun Series* 663–79.
***Eto Y.*** Recent advances in genetic therapies. *Bull Special Formula* 2008; **44:** 1–2.
***Eto Y.*** Renal enlargement in inborn error of metabolism. *J Kid Dialys* 2009; **66:** 161–4.

# Institute for High Dimensional Medical Imaging

Naoki Suzuki, *Professor and Director* Asaki Hattori, *Associate Professor*

## General Summary

The goal of our research is to develop new imaging systems that can be applied to clinical medicine now and in the future. High dimensional, *i.e.,* 3-dimensional (3D) and 4-dimensional (4D), imaging techniques have enabled noninvasive, realistic, uninhibited, and accurate observation 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 medical images by real-time imaging*
We are performing research on the development of medical high-definition image technology and its clinical application using functional and morphological data of a body obtained with X-ray computed tomography (CT) and magnetic resonance imaging.
We are developing a 4D motion analysis system for human activity. An anatomic skeletal muscle model constructed from X-ray CT data sets is driven by motion data obtained with motion capture. This research is being performed by departments in our university in collaboration with Kyushu University, Osaka University, Tsurumi University, and the Mayo Clinic (Rochester, MN, USA).

*Development of an endoscopic surgical robot system*
We are developing an endoscopic surgical robot system that can be used to perform natural orifice transluminal endoscopic surgery. Robotic instruments enter the abdominal cavity orally and are used to perform surgery on the abdominal organs. This year, we modified the system to allow 2 to 4 robot arms to be chosen. We were able to have the robot arms work together to perform surgery. By measuring the pulling force of the wires that drive the robot arms, we tested the functions that would display, to the operator, the softness of the objects that the robot arms grasp. This research is being performed with Kyushu University's department of surgery.

*Development of a simulator for 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 deformation algorithm for grasping abdominal tissue. We modeled the characteristics of the abdominal wall as closely as possible to those of the actual wall and improved the soft tissue model so that reactions when the abdominal wall tissue is grasped and lifted with forceps would be the same as those of the actual wall. This research was performed in collaboration with Kyushu University's department of surgery.

*Development of a surgical navigation system*
We are developing a surgical navigation system that can perform data fusion for 3D images of the interior structure of veins, nerves, or tumors that cannot be seen with the naked eye when surgery is performed under the skin or within organs. This year, continuing a project with our university's department of otorhinolaryngology started last year, we performed microscopic surgery once and stereoendoscopic surgery 3 times in the high-tech navigation operating room of Daisan Hospital.
Moreover, in the "intelligent surgical instruments project," performed in collaboration with Kyushu University, we tested a real-time information integration display system for surgery of the gastrointestinal tract that detects the 3D position and the direction of the tip of an endoscopic surgical system and used the system to perform data fusion of endoscopic images with a patient model.

*Application of the 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, at the request of the Tokyo District Prosecutor's Office, 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. The results were used as evidence in court for the first time.
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**

***Suzuki N, Hattori A, Hashizume M (Kyushu Univ).*** Benefits of augmented reality function for laparoscopic and endoscopic surgical robot systems: augmented medical imaging including augmented reality in computer-aided surgery. *Proc AMIARCS08* 2008; 53–60.
***Suzuki N, Hattori A, Ieiri S[1], Konishi K[1], Maeda T[1], Fujino Y (NTT), Ueda Y (NTT Communications Corp), Navicharern P (Chulalongkorn Univ), Tanoue K[1], Hashizume M[1] ([1]Kyushu Univ).*** Telecontrol of an endoscopic surgical robot system between Japan and Thailand for Tele-NOTES. *Med Meets Virtual Reality* 2009; **17:** 374–9.
***Hattori A, Suzuki N, Otori N, Iimura J, Moriyama H.*** Development of a real-time image-guided surgery system for stereo-endoscopic sinus surgery. *Med Meets Virtual Reality* 2009; **17:** 112–6.
***Ogawa T[1], Ikawa T[1], Shigeta Y[1], Hamada Y[1] ([1]Tsurumi Univ), Hattori A, Suzuki N.*** Designing artificial jaw joints (AJJs) in VR space for patients with rheumatoid arthritis. *Med Meets Virtual Reality* 2009; **17:** 230–2.
***Ikawa T[1], Ogawa T[1], Shigeta Y[1], Fukushima S[1]***

***([1]Tsurumi Univ), Hattori A, Suzuki N.*** The reproduction of high precision 3D maxillofacial reconstruction models. *Med Meets Virtual Reality* 2009; **17:** 125–7.

# Institute of Clinical Medicine and Research

Norio Tada, *Professor and Director*

Akihito Tsubota, *Associate Professor*
Kouichi Nariai, *Assistant Professor*

Sadayori Hoshina, *Associate Professor and Deputy Director*
Yoshihisa Namiki, *Assistant Professor*

## General Summary

The Institute of Clinical Medicine and Research conducts research with a focus on clinical applications. In 2008, the Institute conducted medical research to clarify the etiology, diagnosis, and treatment of intractable diseases. Therefore, we have continued to perform research on the relationship between oxidative stress and disease, including an analysis of the effect of oxidative stress on hepatocarcinogenesis, the role of reactive oxygen species (ROS) in physiological and pathological conditions, and the usefulness of antioxidant agents. We also have performed research to develop the release of anticancer drugs with a magnetically guided nanostructure. Additionally, we have performed studies to define the pathological conditions underlying hyperlipidemia and heart disease. Microbiology has also been a part of our work. The DNA diagnosis of mycosis was performed, and the use of the polymerase chain reaction for rapid, simple, and lower cost testing for epidemiological studies was developed.

## Research Activities

*Liver disease and oxidative stress*

1. Gene expression profiling analysis for oxidative stress-induced liver carcinogenesis

Our group investigated how the continuous exposure to ROS produced in oxidation-reduction (redox) reactions would affect carcinogenesis in the setting of chronic liver damage, using an animal model with naturally occurring and oxidative stress—induced hepatotumorigenesis. On the basis of our experiments, we have narrowed down numerous candidates to 2 signatures. Our gene expression profiling data have been uploaded to the National Center for Biotechnology Information Gene Expression Omnibus website.

2. Development of a novel antioxidant agent

We have verified the antioxidant activity of lactoferrin and proposed its mechanism of antioxidant action. We are attempting to develop a novel type of pegylated lactoferrin and apply it to clinical practice.

3. Analysis of factors contributing to treatment outcome in chronic hepatitis C

To develop more rational and effective treatments for chronic hepatitis C, we are improving antiviral treatment regimens and novel agents by analyzing viral factors, such as kinetics, and host-related factors in cooperation with the Division of Gastroenterology and Hepatology, Kashiwa Hospital.

*Development of drug delivery systems*

The aim of our research is to develop anticancer drug release and magnetically guided nanostructures. To delivery sufficient amounts of therapeutic agents to the interior of deep tumor lesions with minimal drug doses, the combined use of magnetic nanostructures and transplantable magnets is now being developed. We have devised a new self-assembled nanoparticle formulation that can magnetically deliver and silence genes in cells and tumor tissues (Nature Nanotechnology). This work has been supported by an Industrial Technology Research Grant, Program 08C46049a, from the New Energy and Industrial Technology Development Organization of Japan in 2008, by the Futaba Electronics Memorial Foundation in 2008, by the Takeda Science Foundation in 2007, and by the Tsuchiya Foundation in 2006.

*Clinical microbiology*

The DNA diagnosis of mycosis was performed with a morphological base study to assist the Microbiology section of the Central Laboratory and the Rhinosinusitis division of the Department of Otolaryngology. A rapid, simple, and lower-cost test based on the polymerase chain reaction for epidemiological study was developed to replace pulse field gel electrophoresis.

Infectious waste treatment of zoonoses in an experimental animal laboratory was investigated with observation and questionnaires. Guidelines for H1N1 influenza virus waste management were researched with the Ministry of the Environment.

*Induction of follicular regression by photodynamic therapy*

Polycystic ovary is a severe ovarian factor in infertility. Accumulation of follicles without ovulation is peculiarity of polycystic ovary. On the other hand, photodynamic therapy is a physiotherapy that causes cell death through a photosensitizer excited by laser light of a specific wavelength. Photodynamic therapy is used to treat several kinds of cancer. We have found that the photosensitizer accumulates in ovarian follicles. Using this characteristic of the photosensitizer, we examined the induction of follicular regression by photodynamic therapy.

## Publications

***Tsubota A, Satoh K, Aizawa M, Takamatsu S, Namiki Y, Ohkusa T, Fujise K, Tajiri H.*** Four-week pegylated interferon alpha-2a monotherapy for chronic hepatitis C with genotype 2 and low viral load: a pilot, randomized study. *World J Gastroenterol* 2008; **14:** 7220–4.

***Tsubota A, Yoshikawa T, Nariai K, Mitsunaga M, Yumoto Y, Fukushima K, Hoshina S, Fujise K.*** Bovine lactoferrin potently inhibits liver mitochondrial 8-OhdG levels and retrieves hepatic OGG1 activities in Long-Evans Cinnamon rats. *J Hepatol* 2008; **48:** 486–93.

***Kobayashi M, Ikeda K, Arase Y, Suzuki Y, Suzuki F, Akuta N, Hosaka T, Murashima N, Saitoh S, Someya T, Tsubota A, Kumada H.*** Inhibitory effect of branched-chain amino acid granules on progression of compensated liver cirrhosis due to hepatitis C virus. *J Gastroenterol* 2008; **43:** 63–70.

***Koido S, Homma S, Hara E, Mitsunaga M, Namiki Y, Takahara A, Nagasaki E, Komita H, Sagawa Y, Ohkusa T, Fujise K, Gong J, Tajiri H.*** *In vitro* generation of cytotoxic and regulatory T cells by fusions of human dendritic cells and hepatocellular carcinoma cells. *J Transl Med* 2008; **6:** 51.

***Yanai H, Yoshida H, Tomono Y, Hirowatari Y, Kurosawa H, Matsumoto A, Tada N.*** Effects of diacylglycerol on glucose, lipid metabolism, and plasma serotonin levels in lean Japanese.

*Obesity (Silver Spring)* 2008; **16:** 47–51.
***Yanai H, Yoshida H, Tomono Y, Tada N.*** Severe hypoglycemia in a patient with anorexia nervosa. *Eat Weight Disord* 2008; **13:** e1–3.
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***Yokote K (Chiba Univ), Bujo H (Chiba Univ), Hanaoka H (Chiba Univ), Shinomiya M, Mikami K, Miyashita Y, Nishikawa T, Kodama T, Tada N, Saito Y (Chiba Univ).*** Multicenter collaborative randomized parallel group comparative study of pitavastatin and atorvastatin in Japanese hypercholesterolemic patients collaborative study on hypercholesterolemia drug intervention and their benefits for atherosclerosis prevention (CHIBA study). *Atherosclerosis* 2008; **201:** 345–52.
***Yanai H, Tada N.*** A simple hepatic cyst with elevated serum and cyst fluid CA19-9 levels: a case report. *J Med Case Reports* 2008; **14:** 2–329.

## Reviews and Books

***Yanai H, Tomono Y, Ito K, Furutani N, Yoshida H, Tada N.*** The underlying mechanisms for development of hypertension in the metabolic syndrome. *Nutr J* 2008; **7:** 10.
***Tada N.*** Effects of diacylglycerol oil on postprandial increase in serum triglyceride and remnant lipoproteins in humans. In: Katsuragi Y, Yasukawa T, Matsuo N, Flickinger BD, Tokimitsu I, Matlock MG, editors. Diacylglycerol Oil 2nd ed. Illinois, USA: AOCS PRESS; 2008. p. 82–96.

# Medical Engineering Laboratory

Hiroshi Furuhata, *Professor and Director*
Koichi Kanemoto, *Associate Professor*
Masayuki Yokoyama, *Associate Professor*

## General Summary

The Medical Engineering Laboratory has developed new ultrasound (US) therapeutic technologies. This year, with the support of a research grant from the Ministry of Health, Labour and Welfare, we focused on the development of new thrombolytic treatments for patients with acute ischemic stroke. Basic research studies in molecular medical engineering have also been continued to develop an ultrasonic drug delivery system. In particular, the safety of phase-change nanodroplets from fluid to gas by ultrasonication has been evaluated histopathologically, and the increase in nitric oxide (NO) generation by ultrasonication has been analyzed by means of a national database on less-invasive medical devices that includes information on less-invasive technology from around the world and gathers the needs of clinicians. We have also improved previously developed diagnostic techniques for measuring cerebral circulation and hemodynamic variables by the means of noninvasive transcranial ultrasonography. A newly designed research of a less-system combining interdisciplinary diagnostic and therapeutic technology for systematic treatment of acute ischemic stroke was chosen to be a "Super Special Consortia for supporting the development of cutting-edge medical care" by the Ministry of Health, Labour and Welfare.

## Research Activities

*An integrated US system for the diagnosis, analysis, and treatment of acute stroke*
This system uses transcranial ultrasonication to enhance the thrombolytic effects of recombinant tissue plasminogen activator (rt-PA) in the treatment of acute ischemic stroke according to navigation with US performed with a single probe. For the clinical application of this system, we have developed a new device for holding the US probe near the patient's head. Because many Japanese patients have an insufficient temporal bone window, we developed a brain virtual ultrasonography method for use with this sonothrombolysis system in these patients. This new technology can display as a US image in real time the same cross-sectional image obtained with magnetic resonance imaging or computed tomography.

*US thrombolysis*
The accelerating effect on thrombolysis of the combined use of low-frequency (500-kHz) US, rt-PA, and bubble liposomes was verified *in vitro*. Bubble liposomes have a great potential for accelerating the thrombolytic effect of rt-PA with continuous-wave US.

*Verification of the safety of an US drug delivery system for cancer therapy*
We have been developing an US drug delivery system that is integrated with an US diagnostic and therapeutic system with phase-change nanodroplets to provide US images of tumors and to simultaneously kill tumor cells with US heating effects for the selective treatment of tumors. We established a method to verify damage to normal tissue surrounding tumors by means of histopathological evaluation with hematoxyline/eosin and Masson's stain.

*NO generation by US stimulation*
We have reported that an increase in NO generation was found with real-time monitoring when a rat tumor was transcutaneously stimulated with low-frequency ultrasonication.

*Development of a database for less-invasive medical devices*
On the basis of an existing database of nanomedicine, we have developed a new database to provide a "knowledge infrastructure for minimally invasive medical technology." This database can be accessed via the Internet and includes a special forum for discussions of various less-invasive technologies. This development was supported by a research grant from the Ministry of Health, Labour and Welfare.

## Publications

***Shiogai T*[1]*, Ikeda K*[1]*, Morisaka A*[1] *(*[1]*Kyoto Takeda Hosp), Nagakane Y*[2]*, Mizuno T*[2]*, Nakagawa M*[2] *(*[2]*Kyoto Prefect Univ), Furuhata H.*** Acetazolamide vasoreactivity evaluated by transcranial power harmonic imaging and Doppler sonography. *Acta Neurochir Suppl* 2008; **102:** 177–83.

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## Reviews and Books

***Furuhata H.*** Recent sonothrombolysis (in Japanese). *Saishin Igaku* 2008; **63:** 92–104.

# Division of Clinical Pharmacology and Therapeutics

Shigeru Kageyama, *Professor and Director*

## General Summary

The Division of Clinical Pharmacology and Therapeutics was established in July 1995. The aim of the division is to investigate drug treatment, mainly in the area of internal medicine, whereas other departments of clinical pharmacology in Japan focus on registration trials, particularly phase I trials. Because a clinical laboratory where we had performed many human pharmacological studies became unavailable in 2003, we shifted our research from human studies to multicenter clinical trials and pharmacoepidemiological studies.

## Research Activities

We have performed a pharmacoepidemiological study of the prescription of antihypertensive drugs in patients with diabetes. Patients with hypertension complicated by diabetes are considered to be at high risk for cardiovascular events; therefore, the target blood pressure is set lower than for patients who have hypertension but no diabetes, and the drugs of choice are different. We investigated the use of antihypertensive drugs in patients who have hypertension with or without diabetes by means of prescription data from 3 university hospitals.

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 started a pilot study in 3 major hospitals, including our hospital, according to a case-cohort study design in which detailed data were collected in all cases and in a subcohort representing 5% of the whole sample. A full-scale study will be conducted with a large sample size of 20,000 to 30,000 patients.

An important issue for medicine in the 21st century is to identify patients who are responsive or unresponsive and those who show or do not show adverse reactions to drugs. We started a collaborative study with other institutions to examine the relationship between drug-metabolizing enzyme gene polymorphisms and drug effects in residents of an isolated island. We have analyzed the drug-metabolizing enzymes CYP2C9 and CYP2C19. Some of the results have already been applied to drug therapy.

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 (GCP) 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 were 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 applied to be a major clinical trial institution and was accepted. According to this plan, we reinforced CRCs and introduced data managers to improve the clinical trial system. We also introduced an information technology system for processing registration trial management.

## Publications

***Kamijima Y*[1]*, Ooba N*[1]*, Yagame M (Tokai Univ), Samizo K (Bosei Pharmacy), Shimodozono Y (Kagoshima Univ), Kageyama S, Horiguchi S*[1]*, Nagai R*[1]*, Kusunoki T (Jpn Soc Pharmacoepidemiol), Kubota K*[1] *(*[1]*Tokyo Univ).*** Hypertension management in diabetic patients: prescribing trends from 1999 to 2005 in three Japanese unversity hospitals. *Pharmacoepidemiol Drug Saf* 2008; **17:** 904–11.

***Irie S (Kyushu Clin Pharmacol Res Clin), Matsumura Y (Novo Nordisk Pharm), Zdravkovic M*[1]*, Jacobsen LV*[1]*, Kageyama S (*[1]*Novo Nordisk A/S).*** Tolerability, pharmacokinetics and pharmacodynamics of the once-daily human GLP-1 analog liraglutide in Japanese healthy subjects: a randomized, double-blind, placebo-controlled dose-escalation study. *Int J Clin Pharmacol Ther* 2008; **46:** 273–9.

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***Kageyama S.*** Thiazolidinedione derivatives (in Japanese). *Rinshoyakuri* 2009; **40:** 35–9.

***Kageyama S.*** Scientific basis of clinical trials (in Japanese). In: Nakano S, et al, editors. Soyaku ikuyaku iryo staff no tame no rinshoshiken tekisutobukku (in Japanese). Tokyo: Medical Publications; 2009. p. 29–34.

***Watanabe H (Hamamatsu Univ), Kageyama S, Kurihara C (Natl Inst Radiol Sci).*** Actual status of GCP investigations and future perspective:for solution of "over quality issue": Focused on comparation of Japan, US, EU regulations (in Japanese). *Rinsho Hyoka* 2008; **35:** 667–81.

# DDS Institute

Megumu Higaki, *Professor and Director*
Tsutomu Ishihara, *Assistant Professor*
Naomi Yamashita, *Professor*

## General Summary

We are investigating new drug delivery systems (DDS) using nanotechnology. We have developed poly (D, L-lactic acid) (PLA)/poly (ethylene glycol) (PEG)-PLA nanoparticles for targeting and sustained release of steroid/immunosuppressants and have found enhanced anti-inflammatory activity in experimental animal models of arthritis. These studies were partly supported by a Grant from the Ministry of Health, Labour and Welfare of Japan. We also prepared thermosensitive nanoparticles using N-isopropylacrylamide (NIPAAM) to control cellular uptake. The presence of CD208-positive keratinocytes was shown in psoriatic epidermis.

## Research Activities

*Nanoparticle preparations of a steroid for targeting and sustained release*
The purpose of this study was to engineer nanoparticles with various sustained profiles of drug release and prolonged circulation by blending PLA/poly (D, L-lactic/glycolic acid) (PLGA) homopolymers and PEG-block-PLA/PLGA copolymers encapsulating betamethasone phosphate. Nanoparticles of different sizes, drug encapsulation/release profiles, and cellular uptake levels were obtained by mixing homopolymers and block copolymers with different compositions/molecular weights at various blend ratios with an oil-in-water solvent diffusion method. The *in vitro* release of betamethasone phosphate increased with nanoparticles of smaller size or of PLGA homopolymers instead of PLA homopolymers. Furthermore, the uptake of nanoparticles by macrophage-like cells decreased with nanoparticles of higher PEG content, and nanoparticles of PEG-PLGA block copolymers were taken up earlier than those of PEG-PLA block copolymers after incubation with serum. In addition, prolonged blood circulation was observed with nanoparticles of smaller size with higher PEG content, and nanoparticles of PEG-PLA block copolymers remained longer in circulation than did those of PEG-PLGA block copolymers. Analysis of betamethasone phosphate concentrations in organs and *in vivo* fluorescence imaging revealed reduced liver distribution of blended nanoparticles than of PLA nanoparticles. This is the first study to systematically design and characterize biodegradable PLA/PLGA and PEG-PLA/PLGA—blended nanoparticles encapsulating betamethasone phosphate with different release profiles and stealthiness. PLA nanoparticle preparations (about 150 nm in diameter) containing betamethasone phosphate with zinc ion was confirmed to be an appropriate DDS because of the lack of an initial burst. The pharmacological effects by single intravenous injection of this preparation were continued for 1 week in several experimental animal models of inflammation, including rheumatoid arthritis and asthma. The

pharmacological potency of this preparation was 2 to 4 times greater than that of betamethasone sodium phosphate. Because significant accumulation was observed with this preparation in the reticuloendothelial system of the spleen and liver, PEGylation of the nanoparticles has been performed with PEG-PLA block polymers. The anti-inflammatory effects of this stealth-type nanosteroid were 5 to 10 times greater than those of nonstealth nanosteroids in animal models, because stealth nanopartilces escaped from trapping in the liver and specifically accumulated in the inflammatory lesions, possibly helping to reduce the rate of adverse effects.

### Publications

***Ishihara T, Kubota T, Choi T, Higaki M.*** Treatment of experimental arthritis with stealth type PLA/PLGA and PEG-PLA/PLGA blended nanoparticles encapsulating betamethasone phosphate. *J Pharmacol Exp Ther* 2009; **329:** 412–7.

***Ishihara T, Kubota T, Choi T, Takahashi M, Ayano E, Kanazawa H, Higaki M.*** Polymeric nanoparticles encapsulating betamethasone phosphate with different release profiles and stealthiness. *Int J Pharm* 2009; **375:** 148–54.

***Higaki M.*** Recent development of nanomedicine for the treatment of inflammatory diseases. *Inflamm Regen* 2009; **29:** 112–7.

***Ishihara T, Goto M, Kodera K, Kanazawa H, Murakami Y, Mizushima Y, Higaki M.*** Intracellular delivery of siRNA by cell-penetrating peptide modified with cationic oligopeptides. *Drug Deliv* 2009; **16:** 152–8.

***Higaki M, Higaki Y, Kawashima M.*** Expression of CD208 in epidermal keratinocytes in psoriatic lesion. *J Dermatol* 2009; **36:** 136–41.

***Ishihara T, Takahashi M, Higaki M, Mizushima Y.*** Efficient encapsulation of a water-soluble corticosteroid in biodegradable nanoparticles. *Int J Pharm* 2009; **365:** 200–5.

***Ishihara T, Goto M, Kanazawa H, Higaki M, Mizushima Y.*** Efficient entrapment of poorly water-soluble pharmaceuticals in hybrid nanoparticles. *J Pharm Sci* 2009; **98:** 2357–63.

***Ishihara T, Takahashi M, Higaki M, Takenaga M, Mizushima T, Mizushima Y.*** Prolonging the in vivo residence time of prostaglandin E1 with biodegradable nanoparticles. *Pharm Res* 2008; **25:** 1686–95.

# Division of Clinical Research and Development

Satoshi Kurihara, *Professor*
Masato Matsushima, *Associate Professor*
Mitsuyoshi Urashima, *Associate Professor and Director*

## General Summary

Even among patients with the same disease, some may be cured and some may not. This discrepancy cannot be understood through experimental medicine alone. On the other hand, clinical practice might also not provide an answer. For this reason, we combined molecular biology and epidemiology to create the Division of Molecular Epidemiology to clarify the etiology of disease and prognostic factors.

## Research Activities

*The Jikei clinical research course*

From May 2008 through March 2009, we held 2 seminars about strategies for clinical studies for health-care practitioners at The Jikei University. In 2008, small-group study courses targeting postgraduate students were started from the principles of epidemiology and biostatistics by reading textbooks and by analyzing actual clinical data using the STATA software program 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 supplementation to prevent influenza A in school children
2. Activating mutations of epidermal growth factor receptor (EGFR) in squamous cell carcinoma of the head and neck and the *in vitro* response to EGFR antagonists

### Publications

***Kasanuki H, Hagiwara N, Hosoda S, Sumiyoshi T, Honda T, Haze K, Nagashima M, Yamaguchi J, Origasa H, Urashima M, Ogawa H.*** HIJ-CREATE Investigators. *Eur Heart J* 2009; **30:** 1203–12.

***Omi H, Okamoto A, Nikaido T, Urashima M, Kawaguchi R, Umehara N, Sugiura K, Saito M, Kiyono T, Tanaka T.*** Establishment of an immortalized human extravillous trophoblast cell line by retroviral infection of E6/E7/hTERT and its transcriptional profile during hypoxia and reoxygenation. *Int J Mol Med* 2009; **23:** 229–36.

***Eternadmoghadam D, deFazio A, Beroukhim R, Mermel C, George J, Getz G, Tothill R, Okamoto A, Raeder MB, Harnett P, Lade S, Akslen LA, Tinker AV, Locandro B, Alsop K, Chiew YE, Traficante N, Fereday S, Johnson D, Fox S, Sellers W, Urashima M, Salvesen HB, Meyerson M, Bowtell D; AOCS Study Group.*** Integrated genome-wide DNA copy number and expression analysis identifies distinct mechanisms of primary chemoresistance in ovarian carcinomas. *Clin Cancer Res* 2009; **15:** 1417–27.

***Ogi S, Gotoh E, Uchiyama M, Fukuda K, Urashima M, Fukumitsu N.*** Influence of hilar deposition in the evaluation of the alveolar epithelial permeability on 99mTc-DTPA aerosol inhaled scintigraphy. *Jpn J Radiol* 2009; **27:** 20–4.

***Yoshida Y, Goda K, Tajiri H, Urashima M, Yoshimura N, Kato T.*** Assessment of novel endoscopic techniques for visualizing superficial esophageal squamous cell carcinoma: autofluorescence and narrow-band imaging. *Dis*

*Esophagus* 2009 Jan 23. [Epub ahead of print]
***Ishibashi T, Murayama Y, Urashima M, Saguchi T, Ebara M, Arakawa H, Irie K, Takao H, Abe T.*** Unruptured intracranial aneurysms: incidence of rupture and risk factors. *Stroke* 2009; **40:** 313–6.
***Kawasaki N, Suzuki Y, Urashima M, Nakayoshi T, Tsuboi K, Tanishima Y, Hanyu N, Kashiwagi H.*** Effect of gelatinization on gastric emptying and absorption. *Hepatogastroenterology* 2008; **55:** 1843–5.
***Nakayoshi T, Kawasaki N, Suzuki Y, Urashima M, Hanyu N, Yanaga K.*** Epidural analgesia and gastrointestinal motility after open abdominal surgery: a review. *J Smooth Muscle Res* 2008; **44:** 57–64.

# Laboratory Animal Facilities

Kiyoshi Ohkawa, *Professor and Director* Koichi Nariai, *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 2008, 173 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

*Establishment and characterization of the strains derived from the Japanese wild mouse (Mus musculus molossinus) and the Phodopus hamster*

Inbred strains derived by us from the Japanese wild mouse (*M. m. molossinus*) and the *Phodopus* hamster were maintained in the LAF. The Japanese wild mouse originated from a natural intersubspecific hybrid between *Mus musculus castaneus* inhabiting southwest Asia and *Mus musculus musculus* distributed in north Asia. The *molossinus* subspecies is an excellent means for improving laboratory mice, because this subspecies was suspected to be greatly different in genetic constitution from common laboratory mice derived from the *domesticus* subspecies. We have established several new inbred strains based on *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 *molossinus* inbred strain, MSKR. One is a congenic strain having a knockout allele of *Oaz1* derived from the B6.129-*Oaz1*$^{tm}$ 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 research into the genetic modification of *Oaz1* knockout mice. *Phodopus* hamsters are small rodents differing taxonomically from Syrian hamsters, which are the major laboratory hamster. We have recently determined that this hamster is a good candidate for a new laboratory animal and have established an inbred strain. Furthermore, we continue to establish other inbred strains and congenic strains, to develop human disease models, and to study their biomedical characteristics.

*The search for a novel atopic dermatitis therapeutic drug using the NC/Nga inbred strain*

The NC/Nga inbred strain is the current mouse model for atopic dermatitis. However, the rates of dermatitis differ among separate lines at each laboratory. The NC/Nga inbred strain maintained in our laboratory is a line with a particularly severe dermatitis

diathesis. In collaboration with the Department of Tropical Medicine, we are searching for a novel atopic dermatitis therapeutic drug through the use of NC/Nga mice.

*Ovulation inhibition due to removal of peripheral blood phagocytes*

Reactive oxygen species (ROS) containing superoxide are believed to be involved in ovulation. By using a specific superoxide sensor we have recently confirmed the production of superoxide and showed the immunohistochemical localization of DNA and lipid peroxides in the ovulating ovary. Phagocytes, such as neutrophils and macrophages, are thought to be the sources of ROS involved in ovulation. This year, we started to examine whether the removal of peripheral blood phagocytes inhibits ovulation, to examine the source of ROS involved in ovulation.

*Induction of follicular regression by photodynamic therapy*

Polycystic ovary is a severe ovarian factor in infertility. Accumulation of follicles without ovulation is peculiarity of polycystic ovary. On the other hand, photodynamic therapy is a physiotherapy that causes cell death through a photosensitizer excited by laser light of a specific wavelength. Photodynamic therapy is used to treat several kinds of cancer. We have found that the photosensitizer accumulates in ovarian follicles. Using this characteristic of the photosensitizer, we examined the induction of follicular regression by photodynamic therapy.

**Publications**

***Tsubota A, Yoshikawa T, Nariai K, Mitsunaga M, Yumoto Y, Fukushima K, Hoshina S, Fujise K.*** Bovine lactoferrin potently inhibits liver mitochondrial 8-OHdG levels and retrieves hepatic OGG1 activities in Long-Evans Cinnamon rats. *J Hepatol* 2008; **48:** 486–93.

***Ikeshima H, Wada A, Ishiwata K, Watanabe N, Saito S.*** Cloning and expression of cDNA for Interleukin 4 from the MSKR inbred strain of *Mus musculus molossinus*. *In Vivo* 2009; **23:** 277–80.

***Wada A, Ohkawa K, Tsudzuki M (Graduate Sch Biosphere Sci, Hiroshima Univ).*** A candidate gene approach for a black coat color mutation in the *phodopus* hamster. *Exp Anim* 2008; **57:** 466.

***Wada A, Ohkawa K, Tsudzuki M (Grad Sch Biosphere Sci, Hiroshima Univ).*** Sequencing of the tyrosinase gene in the albinotic *Phodopus campbelli*. *Genes Genet Sys* 2008; **83:** 484.

***Motohashi HH, Sankai T, Nariai K, Sato K, Kada H.*** Effects of *in vitro* culture of mouse fetal gonads on subsequent ovarian development in vivo and oocyte maturation *in vitro*. *Hum Cell* 2009; **22:** 43–8.

# Radioisotope Research Facility

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

## General Summary

The Radioisotope Research Facility was established to support medical and biological research with isotopes. We have supported researchers by suggesting methods and practical techniques for experiments. Lectures and training sessions were held for researchers and for medical students and graduate students who are starting to work with radioisotopes. In 2008, 42 researchers from 12 departments consulted this facility for 33 studies.

## Research Activities

*Panton-Valentine leukocidine phages*

The genotypes and Staphylococcal cassette chromosome *mec* (SCC*mec*) types of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) strains have been investigated. With the polymerase chain reaction method, we examined Panton-Valentine leukocidine (PVL)—converting types of 67 MRSA strains isolated in Japan in 1979 through 1985 and in the 2000s and found that 2 morphologically different phages predominated in Japan. The icosahedral head type was identified in 39 of 53 strains isolated from 1979 through 1985. Of the other 26 strains, 25 belonged to the elongated head type. Because the same phage-type strains carry various SCC*mec* elements, the methicillin-sensitive strains lysogenized with the PVL phages likely acquired the SCC*mec* elements independently.

*The second active site of teicoplanin*

The strong synergistic activity of glycopeptide antibiotic teicoplanin with beta-lactams against MRSA suggests an additional mechanism of action. We have speculated that the ATP-binding cassette transporter A is the possible second action point of teicoplanin. Expression of the *abcA* gene was repressed with inducible antisense RNA technique. Downregulation of the ATP-binding cassette transporter A selectively increased the susceptibility to teicoplanin.

*Analysis of the resistance mechanism in radiation resistance organisms*

Tardigrada (*Macrobiotus*) were isolated from mosses growing on the streets around The Jikei University and were irradiated with X-ray at doses of 300 Gy. DNA samples were extracted from Macrobiotus at 5 minutes and 2 hours after irradiation and compared with unirradiated DNA. Agarose gel electrophoresis of the DNAs revealed that Macrobiotus can repair within 2 hours the low molecular weight DNA fragments damaged by X-ray.

*Cherenkov counting of $^{14}C$*

We developed Cherenkov counting of $^{14}C$ with a microplate liquid scintillation counter using a translucent ceramic (Lumicera, Murata Manufacturing Co., Ltd.). The detection efficiency under optimal conditions was 5.0% for the new type-Z ceramic.

*Research on marine bacteria*

The habitat distribution of several marine bacteria was surveyed during *Tansei Maru* cruise KT-08-13. To study the metallic transport system of microbes, we focused on marine bacteria that produce siderophores in a low-iron environment. We are attempting to cultivate such bacteria from seawater and the sediment of the sea floor at a depth of 4,000 m.

**Publications**

***Ma XX*[1], *Ito T*[1], *Kondo Y*[1], *Cho M*[1], *Yoshizawa Y*, *Kaneko J*[2], *Katai A*[3], *Higashiide M*[4], *Li S*[2], *Hiramatsu K*[1] (*[1]Juntendo Univ, [2]Tohoku Univ, [3]Kinan Gen Hosp, [4]Kotobiken Med Lab Inc*).** Two different Panton-Valentine leukocidine phage lineages predominate in Japan. *J Clin Microbiol* 2008; **46:** 3246–58.

# Department of Rehabilitation Medicine
# Division of Physical Fitness

Masahiro Abo, *Professor and Director* Hideki Yamauchi, *Assistant Professor*

## General Summary

The research of our division has been focused on skeletal muscle plasticity, neuroscience, and exercise physiology.

## Research Activities

Myostatin, a member of the transforming growth factor-$\beta$ super family, is a negative regulator of myogenesis and muscle hypertrophy. Peroxisomal proliferator-activated receptor (PPAR) $\gamma$ co-activator 1$\alpha$ (PGC-1$\alpha$) regulates muscular endurance by a shift in fiber type to type I and by mitochondrial biogenesis. We examined the effects of hindlimb unloading with or without intermittent reloading on muscle mass, fiber type, and expression levels of myostatin and PGC1$\alpha$ protein in rat skeletal muscles. We found that the soleus and medial gastrocnemius muscles atrophied by 47% and 31%, respectively, after unloading for 3 weeks. Also, changes in myosin heavy chain isoform composition from type I to IIb occurred in both muscles. The atrophy and shifts to a faster type were ameliorated by resistance exercise. Myostatin expression increased, and PGC-1$\alpha$ expression decreased in both muscles with hindlimb unloading. The resistance exercise restrained these expression changes. We conclude that myostatin and PGC-1$\alpha$ play important roles in the regulation of muscle mass and function.

Receptor activator of nuclear factor kappaB (RANK) ligand (RANKL) is involved in the differentiation and maturity of osteoclasts by coupling with RANK expressed on osteoclasts. We examined the effect of postmenopausal exercise on bone metabolism, including RANKL expression, in ovariectomized rats. The mineral density of the tibia decreased 3 months after ovariectomy. In addition, alkaline phosphatase activity, tartaric acid-resistant acid phosphatase activity, and RANKL protein expression increased with ovariectomy. However, these ovariectomy-induced changes were inhibited by habitual running exercise. We conclude that habitual postmenopausal exercise maintains bone mass by suppressing bone resorption through down-regulation of RANKL expression.

The treatment of obesity is important as an early measure to prevent metabolic syndrome. Adiponectin is secreted from smaller adipocytes and improves insulin resistance. Some studies have found that the effect of weight reduction to increase blood levels of adiponectin is weaker with exercise therapy than with diet therapy. Therefore, we examined the effect of a difference in speed of weight reduction on adipocyte size and blood adiponectin levels in Otsuka Long-Evans Tokushima fatty rats, which have overeating-related obesity.

The results obtained suggest that an exercise program with a low rate of weight reduction

over a longer period is better for maintaining high blood levels of adiponectin than is a program with a high rate of weight reduction over a shorter period.

# Department of Cell Physiology
# Division of Aerospace Medicine

Satoshi Kurihara, *Professor*
Masamichi Sudoh, *Associate Professor*
Hiroko Toshima, *Assistant Professor*

## General Summary

Our main research interests are gravitational physiology and aerospace medicine. We have also studied the relation between human stress and adrenergic receptors (neurons).

## Research Activities

*Optokinetic nystagmus and after-nystagmus during 6 hours of bedrest*

We examined both optokinetic nystagmus (OKN) and optokinetic after-nystagmus (OKAN) with a study in which 6 hours of 6° head-down bedrest was used to simulate microgravity conditions.

In 5 healthy adults, we repeatedly (5 times) and comparatively studied OKN and OKAN evoked by horizontal and vertical stimuli. Stage 1 used an upright sitting position. During the 6 hours' bedrest condition, we studied OKN and OKAN in the 90° recumbent lateral position (stages 2, 3, and 4). For stage 5, the subject was returned to an upright position.

We confirmed that changes in gravity direction had various effects on OKN and OKAN. We also found that more than 3 hours was needed to reach a desirable level of systemic adaptive modification to the unique environmental condition. We believe that the early change was due to changes in sensory inputs through the otolith organs and that the latter changes represent an adaptive process of the spatial orientation system. With tilting, the rates of both horizontal and vertical OKAN decreased; however, the conditions of these changes were different.

A lengthy alteration of gravity direction produced different effects on the intrinsic horizontal and vertical optokinetic oculomotor systems.

*Stress*

1. $Alpha_{2A}$ adrenergic receptors of the platelet membrane: Acute myocardial infarction and cerebral infarction are considered stress-associated disorders. They develop from intravascular microthrombus formation. Therefore, we studied changes in platelet aggregation after stress loading in human subjects.
2. Hypothalamus-sympathetic nervous-adrenal medulla-system: Generally, the heart rate is adjusted by the rhythm of the bulbar circulation centrum. After stress loading, the rhythm is changed through adrenergic neurons that connect the nucleus ceruleus and the medulla oblongata. We then examined the effects of stress on the human circulatory system through frequency analysis of heart-rate variability.

## Publications

***Hida K*[1] (*[1]Nihon Univ), Nomura Y*[1], *Igarashi M*[1], *Sudoh M, Akira T (Japan Aerospace Exploration Agency), Sugiyama Y (Kido Hosp), Shigihara S*[1], *Ikeda M*[1].** Optokinetic nystagmus and after-nystagmus during a 6 hour bedrest study. *Acta Oto-Laryngol* 2008; **128:** 761–7.

***Toshima H, Sugihara H*[1] (*[1]St Marianna Univ), Hamano H*[2] (*[2]Tokai Univ), Sato M (Teikyo Univ), Yamamoto M (Yokohama Stroke Brain Cen), Yamazaki S*[1], *Yamada Y*[1], *Taki M*[1], *Izumi S*[2], *Hoshi K*[1], *Fusegawa Y*[2], *Sato K*[3] (*[3]Yamanashi Univ), Ozaki Y*[3], *Kurihara S.*** Spontaneous platelet aggregation in normal subject assessed by a laser light sacattering method: an attempt at standardization. *Platelets* 2008; **19:** 293–9.

***Sato K (Yamanashi Univ), Shimizu M*[1] (*Tokai Univ), Ohara S*[1], *Goto H (Juntendo Univ), Sano M (Toho Univ), Yamazaki M (Tokyo Women's Med Univ), Toshima H, Yamamoto M (Yokohama Stroke Brain Cent).*** Questionnaire survey on platelet aggregation tests in Japan (in Japanese). *Nihon Kensa Ketsueki Gakkai Zasshi* 2008; **9:** 167–77.

# Department of Orthopaedic Surgery
# Division of Sports Medicine

Keishi Marumo, *Professor* Minoru Shiraishi, *Assistant Professor*

## Research Activities

*Education and 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. The main research topics include:

1) Studies of the efficacy of conservative treatment for acromioclavicular dislocations in athletes.

2) Analysis of specific sports injuries and their treatment in futsal players, based on a year-round experience in medical support for a professional futsal team.

3) Research on medical emergencies, which occur during bicycle road races; studies of year-round medical support and practical problems related to doping control among bicycle road racers.

4) Studies of the characteristics of sports-related injuries in figure skaters.

### Publications

***Funasaki H, Kan I, Kato S, Kasama K, Marumo K.*** Conservative treatment of grade III acromioclavicular dislocations in athletes (in Japanese). *Jpn J Orthop Sports Med* 2008; **28:** 290–4.

# Health Care Center

Mikio Zeniya, *Professor and Director*
Takashi Wada, *Professor*
Kazumi Kawase, *Assistant Professor*
Yoichi Sakamoto, *Professor*
Takekazu Onda, *Associate Professor*

## General Summary

*Shimbashi Medical Checkup Office*

The Japan Society for the Study of Obesity announced the "Kobe Declaration 2006". This "San-san campaign" promoted 3-kg weight reductions or ≥3 cm waist reductions to treat metabolic syndrome. In persons with ≥3-kg weight reduction or ≥3-cm waist reduction, we examined improvements in high blood pressure, dyslipidemia, and glucose intolerance for 1 year.

## Research Activities

*Shimbashi Medical Checkup Office*

In persons with ≥3-kg weight reduction or ≥3-cm waist reduction (n=2,227), we examined improvements in high blood pressure, dyslipidemia, and elevated plasma glucose levels for 1 year.

1. High blood pressure: Subjects were divided into 4 groups according to systolic blood pressures (SBPs) before weight loss: those with SBPs 130 to 139 mm Hg, 140 to 149 mm Hg, 150 to 159 mm Hg, and ≥160 mm Hg. Subjects were divided into 3 groups according to the amount of weight loss: <0 kg, 0 to 3 kg, and ≥3 kg. Reductions in SBP were significantly correlated with reductions in body weight. Among subjects with a systolic blood pressure of 130 to 159 mm Hg before weight loss, 51% achieved a reduction in SBP to <130 mm Hg through a weight reduction of ≥3 kg. In addition, among subjects with a diastolic blood pressure of 85 to 94 mm Hg before weight loss, 67% achieved a reduction in diastolic blood pressure to ≤84 mm Hg.
2. Dyslipidemia: In addition to the above classification of weight change, 58% of subjects with baseline triglyceride levels of 150 to 399 mg/dL achieved reductions to <150 mg/dL through a weight reduction of ≥3 kg. Furthermore, 41% of subjects with a baseline level of high-density lipoprotein cholesterol of 30 to 39 mg/dL achieved an increase to ≥40 mg/dL.
3. Glucose intolerance: Among subjects with a baseline fasting plasma glucose level of 110 to 125 mg/dL, 45% achieved reductions to <110 mg/dL. Compared with changes in waist circumference, body weight correlated well with data improvements. The San-san campaign was easy to remember and useful for treating metabolic syndrome.

At the Harumi Toriton Clinic Medical Check-up office, a new clinical analysis has been started regarding the etiology on metabolic syndrome using serum concentrations of insulin and C-reactive protein.

## Publications

***Oikawa, T, Kamiya A, Kakinuma S, Zeniya M, Nishinakamura R, Tajiri H, Nakauchi H.*** Sall4 regulates cell fate decision in fetal hepatic stem/progenitor cells. *Gastroenterology* 2009; **136:** 1000–11.

***Torisu, Y, Watanabe A, Nonaka A, Midorikawa Y, Makuuchi M, Shimamura T, Sugimura H, Niida A, Akiyama T, Iwanari H, Kodama T, Zeniya M, Aburatani H.*** Human homolog of NOTUM, overexpressed in hepatocellular carcinoma, is regulated transcriptionally by beta-catenin/TCF. *Cancer Sci* 2008; **99:** 1139–46.

***Iwasaki S, Ohira H, Nishiguchi S, Zeniya M, Kaneko S, Onji M, Ishibashi H, Sakaida I, Kuriyama S, Ichida T, Onishi S, Toda G.*** The efficacy of ursodeoxycholic acid and bezafibrate combination therapy for primary biliary cirrhosis: a prospective, multicenter study. *Hepatol Res* 2008; **38:** 557–64.

***Hennes EM, Zeniya M, Czaja AJ, Pares A, Dalekos GN, Krawitt EL, Bittencourt PL, Porta G, Boberg KM, Hofer H, Bianchi FB, Shibata M, Schramm C, Eisenmann de Torres B, Galle PR, McFarlane I, Dienes HP, Lohse AW.*** Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 2008; **48:** 169–76.

# Premedical Course

## Japanese

Ikuko Noro, *Associate Professor*

### Research Activities

*Suitability for patients of informed consent documents written in Japanese*

1. A survey was performed to investigate lay comprehension of readability assessment and the feeling of ease with informed consent documents.
2. A survey was performed to investigate whether medical personnel are able to correctly assess patient comprehension or evaluation or both of an informed consent document.

*Effects of physician gender on medical communication*

1. To investigate the effects of physician gender on medical communication, objective structure clinical examination medical interviews were analyzed with the Roter Interaction Analysis Method.

#### Publications

***Noro I, Muramoto T (Tohoku Univ).*** Lay comprehension of readability assessment and feeling of ease with informed consent documents (in Japanese). *J Jpn Soc Qual Saf Healthc* 2008; **2:** 365–77.

***Noro I, Muramoto T (Tohoku Univ).*** Comparison between patient comprehension and evaluation of an informed consent document and the assessment of the same document by medical staff (in Japanese). *Jpn J Health Behav Sci* 2008; **23:** 120–32.

## Social Science (Law)

Ryuichi Ozawa, *Professor*

### General Summary

Problems of Constitutional Law in present-day Japan

### Research Activities

I address problems of constitutional law in present-day Japan, especially pacifism, parliamentary democracy, public finance, the right of free speech, and the judicial system. I participate in the "Unequal Society and Safety-Net" working party and "Structural Change of Publics" working party of the Legal Committee of the Science Council of Japan.

**Publications**

***Ozawa R.*** Legal strategy of unarmed peace. In: The Law Section of the Association of Democratic Scientists (LSADS), editor. Kaiken Kaikaku to Hou. Tokyo: Nihon-Hyoronsha; 2008. p. 257–62.
***Ozawa R.*** Close-up kenpou. Kyoto: Houritsu-Bunkasha; 2008.
***Ozawa R.*** Political right and democracy of today. *J Jpn Sci* 2008; **43:** 42–7.
***Ozawa R.*** Expricatio and Critik of Soveraign Theories. In: Kainou M, Kurumisawa Y, editors. Kigyou Shijou Shiminshakai no Kisohougakutekikousastu. Tokyo: Nihon-Hyoronsha; 2008. p. 248–63.
***Ozawa R.*** Crisis of Free Speech and Japanese Constitution. Rekishi-Kyouikusha-Kyougikai, Annuals of historic education and sociologic education. Tokyo: Sanseido; 2008. p. 61–72.
***Ozawa R.*** On Legal Meaning of Budget. In: Urata I Tadano M, editors. Gikai no Yakuwari to Kenpougenri. Tokyo: Shinzansha; 2008. p. 163–76.
***Ozawa R.*** Conflict between Freedom of Posting and Safety-Security. In: Mori H, editor. Gendaikeopou niokeru Anzen. Tokyo: Nihon-Hyoronsha; 2009. p. 628–47.
***Ozawa R.*** On Right of Live in Peace. In: Kainou M, Harada S, Hirowatari S, editors. Nihonshakai to Houritsugaku. Tokyo: Nihon-Hyoronsha; 2009. p. 67–83.

# Human Science

Takao Fukuyama, *Professor*

## General Summary

The study of modern German philosophy and ethics

## Research Activities

*Philosophical foundation of behavior change*

From ancient times, philosophy has treated a building of lifestyle (ethos). In our time, the phenomenological approach, founded by Edmund Husserl, treats this theme. According to this phenomenological approach, each man spreads his own meaning world and covers the physical world. This meaning world is named "life-world" by Husserl.

When a disease harms the body of a patient, it also harms, at the same time, the patient's life-world and the patient's mental stability. When medical team intervenes in this situation, their treatment has 2 purposes. First, they cure the illness itself. Second, they must recover the life-world of the patient. The patient restores his reliance on his world. The medical team collaborates with the patient. These processes are the substance of consultation and clinical ethics.

*A requirement of medical ethics*

"How do we live as men?" Traditional ethics has this question answered with the theory of individual happiness. However, this answer is not adequate for medical treatment. The ethics of medical treatment need not be egoistic but should have an altruistic attitude. Medical ethics needs an ethics of responsibility. Viktor Frankl once said, "we must not ask the meaning of our life, the truth is completely the other way

around, to tell the truth, our life itself asks us about the meaning of our behavior". We need to respond to the appeals of others. The sincere attitude to the appeals of others is a requirement of medical ethics.

# English

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

Fujii analyzed the relationships between reading skills and vocabulary knowledge. He also studied example sentences in English learners' dictionaries and found the types of sentences that are conducive to learning. In addition, he developed self-study materials based on the typological analysis of question items found in the Test of English as a Foreign Language.

## Research Activities

Ohara presented a paper at a session entitled "Social Contexts: Chaucer and the *Cely Letters*" at the International Medieval Congress 2008 held at the University of Leeds in the United Kingdom. In this historical sociolinguistic study of the *Cely Letters,* Ohara focused on the usages of auxiliaries and showed how they differ. Ohara visited the National Archive in Britain and obtained digital images of the *Stonor Letters*. Making use of these images, Ohara began his research of the graphemes of the letters of the Stonors.

Fujii presented about the co-relation between reading skills and vocabulary at a conference session of the Japan Society of English Language Education at Showa Women's University in August 2008. He also presented about learner-friendly example sentences in dictionaries in "What Constitutes Good Example Sentences for Dictionary Users and Language Learners ?" at the 15th World Congress of Applied Linguistics in Essen, Germany, in August 2008. In addition, he published a study book for the Test of English as a Foreign Language.

### Publications

***Ohara O.*** What Made Each Writer Write Differently in the *Cely Letters* ? In: Studies in English: The Regional Branches Combined Issue, January 2009. p. 71–83.

***Aizawa K (Tokyo Denki Univ), Yamazaki A (Musashi Inst Technol), Fujii T, Iino A (Seisen Jogakuin Coll).*** The Relationship between Vocabulary Knowledge and Reading Comprehension Skills Used on Reading Tests. In: Annual Review of English Language Education in Japan. ARELE Vol. 20, March 2009. p. 111–20.

### Books

***Hilke R (Hilke Communications, LCC), Wadden P (Int Christian Univ), Fujii T.*** First TOEFL ITP 4 Practice Tests (in Japanese). Tokyo: Alc Publishing Co; 2008.

# German

Yoshiaki Shirasaki, *Associate Professor*

## General Summary

I have continued educational activities for medical students for the purpose of verbal and nonverbal communication. These activities are also connected with the aim of the development of moral and philosophical ability in the field of intercultural relationships. Per year, 120 hours of activities are held.

### Publications

***Shirasaki Y.*** The sense of selfless fairness. In: Zum roten Egel. The Yearbook of Japanese Brahms Society in Tokyo December, 2008. p. 87–8.

***Shirasaki Y.*** Moerikes Novel Maler Nolten as a Realismusroman, Auf der Jahrestagung der Japanischen Gesellschaft für die deutsche Literatur des 19. Jhd. in Osaka, 6. December. 2008.

# Mathematics

Kanji Suzuki, *Professor*

## General Summary

We have considered and improved the methods of teaching calculus. Continuing last year's proposal at regular meetings of the Mathematics Education Society of Japan, we have proposed a method for teaching the theory of linear differential equations with constant coefficients.

## Research Activities

For students in the early years of colleges of technology or medicine, it is not easy to understand the concepts of linear independence on several functions and of the

Wronskian on them.
Instead of using these concepts, we adopt the fact that for mutually prime polynomials $f_1(t), \cdots, f_n(t)$, there exist polynomials $g_1(t), \cdots, g_n(t)$ such that $\sum_{i=1}^{n} g_i(t)f_i(t)=1$ (an identical equation).
Using this property, we can develop a general theory of linear differential equations with constant coefficients without a leap of logic.

#### Publications

***Suzuki K.*** Linear differential equations with constant coefficients: teaching methods without using Wronskians (In Japanese). Tokyo: Mathematics education society of Japan (a special edition); 2008.

# Physics

Koichi Satoh, *Professor*

Katsumi Kasono, *Assistant Professor*

## General Summary

1. Dipalmitoylphosphatidylcholine (DPPC) membranes have been studied from several points of view, including form formation, liquid crystal, phase transitions, interaction with ions, and optical characteristics.
2. Phase transitions, critical phenomena, interacting many-body systems, and computer simulation.

## Research Activities

*Ripple phase of the DPPC membrane*
We have studied the difference between unilamellar and multilamellar liposomes in the ripple phase and the consistency of our ripple phase model in several phenomena.

*Monte Carlo simulations of the $q=10$ Potts model*
We have made cluster update simulations to the study of systems with the first-order phase transition. We calculated latent heat. The result was consistent with the theoretical prediction to 3 digits.

# Chemistry

Takashi Okano, *Professor* Chikao Hashimoto, *Associate Professor*

## General Summary

The research of this laboratory involves synthesis-oriented organic chemistry, including synthesis of bioactive compounds, synthesis of fluorine-containing materials, and the development of new methods for peptide synthesis; and computer-assisted analysis of materials and synthetic reactions.

## Research Activities

*Molecular design of gadolinium (III) sugar ball complex for a highly sensitive magnetic resonance imaging agent*

Gadolinium (III) heterocyclic complexes are used as magnetic resonance (MR) imaging agents because of the high spin multiplicity to catalyze spin-spin relaxation of exited water protons. Modification of the heterocyclic ligand with gluconic acid moieties improves imaging sensitivity and decreases the needed dose of toxic imaging agents. We performed computational analysis of the mechanism of spin relaxation by the gadolinium (III) complex and the related manganese (III) complex, and examined the appropriate structure of the gluconamide-modified gadolinium (III) complexes using density functional theory, semiempirical molecular orbital theory, and molecular mechanics techniques.

*Synthesis of N-protected peptide acids using amino acid-alkaline earth metal salts*

The protection of a carboxyl group by a metal ion decreases the time needed for the incorporation and removal of the protecting group and prevents side reactions caused by the use of esters. The syntheses of N-protected peptide acids in organic solvents using alkaline earth metal-carboxylate salts of an amino acid were investigated. We found that the amino acid-Ca carboxylate salts are the most effective carboxylate salts of amino acid tested for coupling with Boc-amino acid activated esters in organic solvents, such as dimethylformamide and dimethylsulfoxide.

# Biology

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

*New pattern of phragmoplast growth brings about asymmetric cell division in pollen of Ephedra*

Pollen of *Ephedra* form as male gametophytes by undergoing asymmetric cell division five times following meiosis, going through the discrete stages of microspore, embryonal cell, antheridial initial cell, generative cell and body cell. The asymmetric cell division of the first four of these cell types was shown to involve eccentric location of the nucleus and mitotic apparatus at one end of the longitudinal axis of the ellipsoidal cell.

Furthermore, a previously unknown growth pattern of the phragmoplast was shown to increase the inequality of division of the embryonal cell, antheridial initial cell and generative cell; this anomalous growth was particularly noticeable in division of the antheridial initial cell. At early telophase of antheridial initial cell division, the phragmoplast is made up of two rings of microtubules of equal diameter, arranged symmetrically about the plane of division; these grow centrifugally between the two daughter nuclei, perpendicular to the axis of division. Before the rings reach the lateral membrane of the mother cell, their relative diameters change and they take on a concentric arrangement. The direction of their growth changes, becoming parallel to the axis of division so that the phragmoplast surrounds the generative cell nucleus as it grows. At late telophase, the two sets of rings return to their symmetrical arrangement, and as they reduce their diameter they reach the cellular membrane at one end of the longitudinal axis of the cell, and asymmetric cell division is complete. In body cell division the phragmoplast also undergoes initial development between the two daughter nuclei, but subsequently degenerates without forming a cell plate, so that a single sperm cell forms with a large and a small nucleus.

# School of Nursing

## Basic Nursing I

Sawako Haga, *Professor*
Mayumi Kikuchi, *Assistant Professor*
Machiko Hirao, *Associate Professor*
Chieko Hanyu, *Assistant Professor*

### Research Activities

The research activities of the basic nursing group can be divided into the following 3 areas.
Haga has been investigating the effects of physical assessment by nurses, the evidence of nursing skill, and the history of nursing.
Hirao has been investigating the history of nursing and Nightingale's thoughts about nursing.
Kikuchi has been investigating the effects of nursing education, methods of teaching, and nursing diagnosis.
Hanyu has been investigating the effects of physical assessment by nurses and the evidence of nursing skill.

#### Reviews and Books

***Hirao M, Haga S, Ebina F.*** A study of Dr. Kanehiro Takaki's thought on health education (3): from his opinions related to the improvement of teacher's education and vocational education In "Rinji Kyouiku Kaigi" (in Japanese). *J Jpn Soc Med Hist* 2008; **54:** 119.
***Hirao M.*** Dr. Billroth's nursing book which was translated in Japanese and published in 1895 (in Japanese). *J Jpn Soc Med Hist* 2008; **54**: 76.
***Oishi S, Hnyu C.*** Import of foreign nursing. In: Hirao M, Nursing Academic Society, Japan Society of Nursing History, editors. Nursing in Japan in the past 120 years: to pioneers making the history of nursing. Kangokyoukai syuppannkai (in Japanese). 2008. p. 121–3, 127–30.
***Koizumi J, Kikuchi M, Takahashi Y, Takahara S, Nakafuji M, Kuroda Y, Tuda Y, Saitou A, Sugita R, Simomai K.*** Identification of the charactaristic that nurses perceive human response as "Sense of abdominal fullness"(1) (in Japanese). *J Nursing Diagn* 2008; **14:** 161–2.
***Kikuchi M, Takahashi Y, Takahara S, Nakafuji M, Koizumi J, Tuda Y, Saitou A, Sugita R, Simomai K, Kuroda Y.*** Identification of the charactaristic that nurses perceive human response as "Sense of abdominal fullness"(2) (in Japanese). *J Nursing Diagn* 2009; **14:** 163–4.
***Takahashi Y, Kuroda Y, Yamada A, Tuda Y, Simomai K, Kikuchi M, Koizumi J, Nakafuji M, Sugita R, Takahara S.*** Identification of the charactaristic that nurses perceive human response as "sense of abdominal fullness", the investigation of the initial stage to develop "sense of abdominal fullness" (in Japanese). *J Nursing Diagn* 2009; **14:** 15–26.
***Egawa A, Hnayu C, Nakajima S, Suwa K, Anazawa S.*** Stoma management of 1 ocal condition (in Japanese). *J Jpn Soc Stoma Continence Rihabil* 2009; **25:** 129.

# Basic Nursing 2

Sugino Oishi, *Professor*

## General Summary

To clarify the characteristics of the Japanese nursing system after World War II, I have been studying the history of nursing and analyzing the factors affecting the Japanese nursing system.

## Research Activities

*Quantitative analysis of the changes in the number of nursing graduates and assistant nursing graduates in Japan from 1974 to 2006*

The purposes of this study were (1) to analyze changes in the number of nursing graduates (from nursing universities, nursing colleges, or nursing schools), the number of assistant nursing graduates (from assistant nursing schools or nursing high schools), the ratio of the number of nursing graduates to that of the number of nursing graduates and assistant nursing graduates (nursing graduation ratio) and (2) to analyze factors that caused the changes from 1974 to 2006 in Japan. For these analyses, official data were used.

*Study of the policy of the Nursing Affairs Division GHQ by analyzing censorship by GHQ*

Records of the censorship of medical and nursing publications from 1945 through 1949 in occupied Japan are thought to be housed in the Gordon W. Prange Collection at the University of Maryland.

The purpose of this study was to investigate the circumstances of publishing and the criteria for censorship by General Headquarters Supreme Commander for the Allied Powers (GHQ) in occupied Japan. I visited the Gordon W. Prange Collection and collected and analyzed its "Pamphlet List" in 2008.

*Historical research on Japanese nursing reform after World War II*

The Nursing Affairs Division of GHQ revised the nursing law and the nursing system during the occupation. The present Japanese nursing system is based on the nursing reforms of the GHQ but has been modified because of economic and educational problems. I obtained information from the GHQ records and related persons to investigate nursing reform.

I introduced the methods of historical investigation and presented the results of my study to students in my lecture on nursing management.

### Publications

***Oishi S.*** History of hospitals in Japan after World War II (in Japanese). *Hospital* 2009; **68:** 65–8.
***Oishi S.*** Evaluation of nursing reforms after World War II (Establishment of the Nursing Division in the Ministry of Health and Welfare (in Japanese). *J Jpn Soc Med Hist* 2008; **1530:** 124.

### Reviews

***Kawashima M, Tanaka S, Oishi S.*** Nursing History in Japan after World War II (in Japanese). *Kango* 2008; **60:** 68–77.
***Oishi S, Hanyu C.*** Import of foreign nursing (in Japanese). Nursing in Japan for 120 years. Tokyo: Japanese Nursing Association Publishing Company; p. 115–32.
***Yoshikawa R, Oishi S.*** Nursing during the war (in Japanese). Nursing in Japan for 120 years. Tokyo: Japanese Nursing Association Publishing Company; p. 165–82.

# Adult Nursing

Shoko Fujino, *Professor*
Chie Watanabe, *Assistant Professor*
Ryuko Fujimura, *Professor*

## General Summary

We have studied the acquisition of nursing skills in clinical adult practice. We examined nursing skills after clinical practice in adult nursing.
We examined what nursing skills graduates had wanted to study while they were students at our university. Many nurses wanted to learn how to dispense medicines to patients and to manage drip infusions. We increased the number of classes needed to teach these skills.

## Research Activities

Fujino studied the effects on palliative care of touch techniques by nurses. We 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 the touches were comforting and touched patients to comfort them. We have called their use of touch "caring touch" in reference to the relationship between nurses and patients to relieve suffering.
Watanabe developed guidelines on nursing care for ambulatory chemotherapy. We have also performed an interventional study to evaluate the feasibility and validity of these guidelines on nursing care for ambulatory chemotherapy.

### Publications

***Shouji D[1] ([1]Cancer Inst Hosp), Matsusaka S[1], Watanabe C, Suenaga M[1], Shinozaki E[1], Matsuda M, Kuboki Y[1], Itimura T[1], Ogura M[1], Chin K[1], Mizunuma N[1], Hatake K[1].*** Relative dose intensity of FOLFOX4 therapy for recovery from advanced colorectal cancer (in Japanese). *Jpn J Cancer Chemother* 2008; **35:** 1895–900.

**Reviews**

***Fujino S.*** Health Teaching for Community Person (in Japanese). Adult Nursing. 2nd. Tokyo: Nouvelle Hirokawa; 2009. p. 309–21.
***Watanabe C.*** Sexuality after hematopoietic stem cell transplantation (in Japanese). Improvements of quality of life after hematopoietic stem cell transplantation. Osaka: Iyaku Journal sha; 2008. p. 44–8.
***Watanabe C.*** Counseling about sexuality (in Japanese). Basics and clinical medicine regarding hematopoietic stem cell transplantation. Osaka: Iyaku Journal sha; 2008. p. 315–9.
***Watanabe C.*** Cancer for women (in Japanese). Women's Health. 2nd Tokyo: Medical frend sha; 2008. p. 244–51.
***Watanabe C.*** Cervical cancer and care (in Japanese). Women's Health. 2nd. Tokyo: Medical friend sha; 2008. p. 250.
***Watanabe C.*** Endometrial cancer and care (in Japanese). Women's Health. 2nd. Tokyo: Medical friend sha; 2008. p. 251–3.
***Watanabe C.*** Breast cancer and care (in Japanese). Women's Health. 2nd. Tokyo: Medical friend sha; 2008. p. 254–8.

# Gerontological Nursing

Miyoko Sakurai, *Professor*

Kumiko Date, *Associate Professor*

## General Summary

In the field of gerontological nursing, we have studied effective educational methods, such as lectures and clinical training, from the perspective of the quality of life of elderly persons.

## Research Activities

*Educational methods for preventing bedsores in the elderly*
We experimented with an educational method that provides visual hands-on training with a pressure-mapping system for bedsore prophylaxis in the elderly. This year, the training method was evaluated. The findings suggested that students who underwent visual hands-on training had a deeper understanding of the appropriate nursing skills for preventing bedsores.

*Research into the psychology of families of elderly persons with dementia*
Sakurai et al. have been investigating the psychological conflicts of family caregivers of elderly persons with dementia. This year, we performed interview surveys of family caregivers in the community.

*Relationship between health and lifestyle in elderly adults*
Date has been investigating the health status and various factors influencing it among middle-aged and elderly adults for the primary and secondary prevention of lifestyle-related diseases from a comprehensive perspective, including nutrition, exercises, and rest.

**Reviews and Books**

***Date K.*** Management and education of the enteral and parenteral nutrition (in Japanese). Nutrition sciences for clinical and home care. Tokyo: Nouvelle-Hirokawa; 2009. p. 249–74.

# Mental Health and Psychiatric Nursing

Masashi Kawano, *Professor* Setsuko Hayashi, *Assistant Professor*

## General Summary

Educational contents need to be evaluated because of the 21st nursing curriculum revision. To teach updated educational contents, we must develop and renew both educational methods and educational materials. We must also attend conferences and perform research. Next year maters program will open time control will be needed.
Kawano made a DVD contents were psychiatric nursing care with staff members of a clinical practicum hospital. Efforts continued to enhance the collaborative relationship with a clinical practicum hospital. This year a needed expansion was started of "conference community collaborative psychiatric mental health nursing."
To develop educational methods, the learning experiences of students participating in laboratory study and clinical practicums were analyzed. In a clinical nursing wisdom in Morita Therapy is workable in education, it is important to test its educational effects.

## Research Activities

Professor Kawano started working this year, and an assistant professor and a lecturer were added to the faculty.
Kawano is conducting research on the child and adolescent mental health support network, mainly in Kanagawa prefecture. Results were presented at several conferences, published in a book and a journal, and given in a classroom lecture.
Hayashi focused on psychiatric patients' process for informed and consent when nurses explain nursing care plans to patients. Nurses were interviewed to clarify their thoughts and conflicts. Nurses make efforts to reach a agreement with patients. Through interviews of nurses working at the Morita Therapy Center, the recognition of nursing care at the Morita Therapy Center was examined. It makes clear that nurses need not to Toraware for patients not to Toraware in their in-patient life.
For students' clinical practicum, contract needed to be made with a new psychiatric hospital, and agreement needed to be reached with Kichijoji Hospital. Kawano is making a DVD with the members of the staff of Kichijoji Hospital.

# Child Nursing

Kiyo Hamanaka, *Professor* Kayo Cho, *Associate Professor*

## Research Activities

*Development and verification of an educational support program to continue working in child care*

Hamanaka held a training seminar based on a tentative plan to help nursing students continue working after graduation. Hamanaka received feedback from participants and clarified the effects and problems of the program.

*A study of the organization of practice in nursing with outpatients and outpatient nursing to promote the health of children in basic education*

As a co-author of a study with the support of a Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Hamanaka presented results of a study from last year at a congress and chaired a workshop at the annual meeting of the Society of Ambulatory and General Pediatrics of Japan.

In addition, Hamanaka carried out a program that was developed last year for outpatients and collected data from children aged 4 to 6 years, their parents, and nursing students.

*The social growth process of children with end-stage renal disease and drafting a support plan by offering information*

Cho interviewed children with end-stage renal disease and their parents to clarify methods to deal with problems that patients and parents face in the process of social growth. Cho presented results at the 30$^{th}$ congress of the Japanese Society for Pediatric Renal Failure.

# Maternity Nursing

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

*The relationship between menstrual symptoms and mind-body characteristics in adolescent girls*

This study of 323 adolescent girls analyzed the relationships among physique, lifestyle, health attitudes, gender-role perception, and other factors to clarify mind-body factors related to menstrual symptoms. Approximately 80% of the target group was of average build, but half perceived themselves to be overweight. An analysis of the relationship with menstrual symptoms (Menstrual Distress Questionnaire) revealed significant correlations with body-mass index ($p<0.05$), family factors ($p<0.05$), and chance factors ($p<0.05$) in regards to the subjective sense of health control (Health Locus of Control Scale) in premenstrual high school students and a significant correlation with chance factors on the Health Locus of Control Scale ($p<0.05$) for students in the middle of the menstrual cycle. Among university students, there was a significant correlation with negative attitudes toward menstruation ($p<0.01$), self-care ($p<0.05$), and androgyny ($p<0.01$) in regards to gender role personality (Bem Sex Role Inventory) in both students who were premenstrual or in the middle of the menstrual cycle.

*Sexuality issues and nursing care*

1. Nursing and sexuality: Nursing in the context of an induced abortion case

Cases involving induced abortion are cited as an issue concerning sexuality which is encountered in a nursing setting. A brief account is given of the nurse's assessments, care points, and support for the family, taking into account biological, psychological, and sexual sequelae resulting from an induced abortion.

2. Study of touching during counseling with the opposite sex

Case studies were examined to examine the appropriateness of nurses touching patients of the opposite sex during counseling. Because touching carries the risk of evoking sexual feelings if the nurse and patient are of the opposite sex and is either taboo or should be undertaken with prudence, further study is needed in this area.

*Investigation of the future direction of midwifery education at universities*

As a result of the creation of a specialized graduate school at Tenshi College in 2004, midwifery education has shifted toward having the instruction provided after the

conclusion of undergraduate studies in a graduate school, university major course of study, or special course. Reasons that establishing such a graduate school, university major course of study, or special course is difficult are that the fees required for midwifery education, differences of opinion about midwifery education, and securing instructors are considered extremely controversial issues. Economic support from the government for midwifery education is expected in the future.

*Investigation of university instructor satisfaction and related factors*
We are preparing a study of satisfaction among university instructors engaged in practical training for maternity nursing, as well as relevant factors. The study will focus on maternity nursing instructors at 4-year universities in Japan, and preparations are being made to nursing university teachers' self-efficacy for nursing practice education, along with a simple questionnaire on occupational stress.

**Reviews and Books**

***Kawano M, Kayashima K, Otani M (Chiba Coll Health Sci).*** Nursing and sexuality—The one —Nursing of the artificial abortion (in Japanese). *Jpn J Sexol* 2008; **26:** 73–76.

# Community Health Nursing

Noriko Okuyama, *Professor* Miki Shimada, *Associate Professor*

## General Summary

The major research projects in our department have been focused on: 1) the learning achievements of public health nurses and 2) establishing a community-based end-of-life care system through the organization of social capital.

## Research Activities

*Public health nursing education*
This study aimed to develop an essential skills framework and the achievement levels necessary for students graduating from schools that provide basic education for obtaining a license as a public health nurse in Japan.

*Establishing a community-based end-of-life care system through the organization of social capital*
The purpose of this study was to identify the components necessary to establish a care system, such as a mutual regional support mechanism, understanding by the general public, and network structures, focusing on social capital, to play a role in the establishment of a community-based end-of-life care system.

# Home Care Nursing

Hiromi Kasuga, *Assistant Professor*

## General Summary

Home care nursing is the field that studies patients who receive medical care at home, families who care for patients, and the nursing support needed by patients and families.

## Research Activities

*Research on an e-learning system for students of nursing*
To plan a seminar and practicum effectively in a limited time, we used an e-learning system, as a self-learning tool. We obtained information about outcomes with this system for students of nursing. In future studies, we will explore the possibility of using this system in various situations for studies of home care nursing.

*Research about patients who die of cancer at home and their families*
We investigated the cost of care for patients with cancer who die at home, the feelings of the family of the deceased, and the support visiting nurses provided to the family.

*Research about the professional skills of nursing staff who engage in the visiting bath service (in Japanese)*
Bath service nurses observe the physical condition of patients as the only medical staff involved in home bath services. We investigated the experiences of nurses attending patients who receive bath service in their home.

*The research about nursing support for patients when patients are discharged from hospitals to their home (in Japanese)*
We investigated nursing services that are required by patients who were discharged from hospitals to their homes. This year, we investigated the difficulties of nurses when they guide elderly patients in medical treatment at home.

### Publications

***Kasuga H, Sato M, Toyama H.*** What is needed with the professional skills of the nursing staff who engage in the visit bath service (in Japanese). *Jpn J Nurs Assoc* 2008; **38:** 151–3.
***Kasuga H, Sato M, Toyama H.*** The expense of home care for terminal cancer patients and their bereaved family's feeling for payment (in Japanese). *Jpn J Health Sci Res* 2008; **12:** 51–7.

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