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Introduction

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

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

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

Senya Matsufuji
President
The Jikei University School of Medicine

December 12, 2013

Continuing Medical Education Center

The Continuing Medical Education Committee

Toshiaki Abe, *Director*
Yashuo Toriumi
Rimei Nishimura

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 physicians outside the university hospital. Registered members consist of alumni throughout Japan, members of the local medical association, and physicians who have been approved by the Jikei CME Center. Members are allowed to use the facilities (videos, library) of the Center and other facilities (medical library, medical museum) of the university. A telephone service is available at all times. Members may also attend or participate in summer and monthly seminars sponsored by the Center and in scientific meetings and conferences held by the department.

Activities

1. Registered members: 222 (as of April 1, 2013)
Members using the Center: 114/year
Telephone service: 75 cases
2. The 33rd summer seminar was held on August 4, 2012. Seventy-six 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, *Professor and Director*
 Mariko Itsubo, *Professor*
 Toshikazu Sakuyama, *Associate Professor*
 Masato Matsushima, *Associate Professor*
 Nobuyuki Furutani, *Associate Professor*
 Yoshio Ishibashi, *Assistant Professor*

Naofumi Kimura, *Professor*
 Tetsuya Kawamura, *Associate Professor*
 Hisashi Onoue, *Associate Professor*
 Mariko Nakamura, *Associate Professor*
 Hiroyuki Takahashi, *Associate Professor*
 Fumiko Okazaki, *Assistant Professor*

General Summary

The Office of Educational Development was founded in 1999. Staff members were recruited from the School of Medicine. Its main interests were the analysis of medical education reports published by the Ministry of Education, Culture, Sports, Science and Technology (MEXT); the Ministry of Health, Labour and Welfare; and medical associations; technical support of faculty and management of faculty development and education seminars; and the implementation of tutorials, objective structured clinical examinations (OSCEs), and community-based medical education programs in the undergraduate curriculum. However, many improvements have been required in our undergraduate medical and nursing education, postgraduate clinical training programs, and continuing professional development for healthcare workers. In 2005, the office of Educational Development was reorganized as the Center for Medical Education. Furthermore, the secretariat was set up in the Center in 2006. The bylaws of the Center were established in 2011. The Center now consists of the Branch for Medical Education and the Branch for Nursing Education (the Center for Nurse Career Support). The branches contribute to undergraduate educational activities in the medical and nursing schools and practical nursing schools, staff development in the university and the 4 attached hospitals, and the management of e-learning systems and simulation training centers for students, faculty, and staff in attached hospitals and healthcare providers in the community.

Research Activities

1. Our proposal, “Establishing systematic medical education for implementing clinical clerkships,” was selected by MEXT to receive a Supporting Grant for Improving Clinical Clerkships According to a Global Standard for Medical Education Program 2012. This project aims to expand clinical practice education to 74 weeks (62 weeks at present) and to extend locations for students’ clinical experience to affiliated hospitals. The project also proposes changes in the preclinical educational period: patient contact from the early years and learning basic sciences in a clinical context. King’s College London will support our curriculum reform.
2. A cooperative proposal with Tokyo Medical and Dental University, Tokyo University, Niigata University, Chiba University, and Tokyo Women’s Medical University, “Establish an accreditation system for basic medical education compliant to global

standards,” was selected by MEXT to receive a Supporting Grant for Improving Clinical Clerkships According to a Global Standard for Medical Education Program 2012. In the collaborative project, we did research on an accreditation system in the United Kingdom. The system is administered by the General Medical Council and in 2010 changed from a comprehensive approach to a risk-based approach. Furthermore, we started to collect data on educational outcomes in the School of Medicine for making a self-evaluation form as a first step of the accreditation process.

3. We promoted an Innovative University Education project supported by MEXT called “Research for Changes in Healthcare Education according to the aging of the population.” In this project, we investigated educational outcomes of patient-contact programs in the community. Since 1999 we have included in-home care practice in year 3. We qualitatively analyzed students’ descriptions of their learning outcomes. Students learned about home-care, teamwork among healthcare providers in the community, and the roles of physicians in in home care.

4. We promoted “Research on Nurse Practitioners Working with Cancer Specialists (physicians and pharmacists)” with a Supporting Grant for Clinical Cancer Research 2010 from the Ministry of Health, Labour and Welfare. We investigated Japanese physicians’ attitudes regarding the education of nurse practitioners by sending surveys to specialists (members of the Japan Society of Clinical Oncology) and generalists (members of the Japanese Primary Care Association and the directors of clinical training programs for physicians). We found that cancer specialists expected nurse practitioners to provide symptom management and psychosocial support, clarify information, provide education, and work as a member of a multidisciplinary team.

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

6. Contribution to other institutions of higher education (faculty development lectures and workshops): Ibaragi Nursing Education Association (May), Showa University (July), Hyogo Medical College Hospital (July), Kyoto Practical School for Healthcare (August), Fuchu Practical School of Nursing (August), Tokushima University Hospital (September), Hyogo Medical College (September), Yokohama City University (September), Otaru Ekisaikai Hospital (October), Itabashi Chuo Sougou Hospital (October), Iwate Medical University (November), Yamaguchi University (November), and Daito Bunka University Faculty of Law (February)

Publications

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Okazaki F, Furutani N, Seki M, Ishibashi Y, Onoue N, Ono K, Akiyama M, Kawamura T. Effects of a workshop for clinical clerkships at The Jikei University (in Japanese). *Igaku Kyoiku*. 2012; **43**: 441-6.

Department of Anatomy (Gross Anatomy and Neuroanatomy)

Yoshinori Kawai, *Professor*

Toru Hashimoto, *Assistant Professor*

General Summary

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

Research Activities

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

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

Local axonal arborization patterns of distinct neuronal types in the cNTS

Neurons in the cNTS are heterogeneous in size (50 to $450 \mu\text{m}^2$ in somal area) and other morphologic characteristics. For a more objective classification of cNTS neurons,

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

Postnatal development of GABAergic axon terminals in the rat cNTS

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

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

Neural activity during critical periods could fine-tune functional synaptic connections. The activation of *N*-methyl-D-aspartate (NMDA) receptors is critically implicated in this process, and blockade leads to the disruption of normal circuit formation. This phenomenon has been well investigated in several neural

systems, including the somatosensory system, but has not yet been evidenced in the visceral sensory system. Ultrastructural analysis of GABAergic synapses and electrophysiological analysis of inhibitory and excitatory postsynaptic currents of cNTS cells revealed that developmental changes in the synaptic organizations were blocked by MK-801, an NMDA receptor antagonist, when administered at P5 to P8, a presumed critical period for the visceral sensory system. Normal synapse reorganization during postnatal development dictates undifferentiated neonatal cNTS neurons in terms of synaptic input patterns measured with electron microscopy and electrophysiologic studies into 2 cell groups: small cells and large cells under far stronger excitatory and inhibitory influence, respectively. Blockade by MK-801 during the critical period might leave adult neurons wired in the undifferentiated synaptic networks, possibly preventing synapse elimination and subsequent stabilization of the proper wiring.

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

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

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

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

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

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

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

Geometric and functional architecture of visceral sensory microcircuitry

Is microcircuit wiring designed deterministically or probabilistically? Does geometric architecture predict functional dynamics of a given neuronal microcircuit? These questions were addressed in the visceral sensory microcircuit of the cNTS, which is generally thought to be homogeneous rather than laminar in cytoarchitecture. By means of in-situ hybridization histochemistry and whole-cell patch-clamp recordings followed by neuronal reconstruction with biocytin filling, the anatomical and functional organization of NTS microcircuitry was quantified to determine associative relationships. Morphologic and chemical features of NTS neurons showed different patterns of process arborization and subnuclear localization according to neuronal type: smaller cells featured presynaptic local axons, and GABAergic cells were aggregated specifically within the ventral NTS. The results suggest both a laminar organization and a spatial heterogeneity of NTS microcircuit connectivity. Geometric analysis of the presynaptic and postsynaptic axodendritic arbor overlap of reconstructed neurons (according to parent somal distance) confirmed a heterogeneity of microcircuit connectivity that could underlie the differential functional dynamics along the dorsoventral axis. Functional dynamics in terms of spontaneous and evoked postsynaptic current patterns behaved in a strongly location-specific manner according to the geometric dimension. This finding suggests a spatial laminar segregation of neuronal

populations: a dorsal group of high excitation and a ventral group of balanced excitation and inhibition. Recurrent polysynaptic activity was also noted in a subpopulation of the ventral group. Such geometric and functional laminar organization seems to provide the NTS microcircuit with both reverberation capability and a differentiated projection system for appropriate computation of visceral sensory information.

Department of Anatomy (Histology and Embryology)

Masataka Okabe, *Professor*
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 the human body. By comparing organ development among vertebrates, we are attempting to reconstitute the evolutionary path that each of our organs has taken, at both the molecular and morphological levels, thus identifying fundamental molecular mechanisms that shape each organ.

Research Activities

Histopathological changes in the peripheral nervous system of the novel ataxia mouse before the onset of ataxia

The progressive ataxic gait mouse (Ataxic mouse) developed in our laboratory has hind limb ataxia that manifests from about 4 weeks of age and becomes more severe with age. Histopathological investigations of the Ataxic mouse showed vacuolar degeneration in the medulla of the cerebellum, the spinal nerves, and the trigeminal nerve. These disorders are inherited in an autosomal recessive manner, but a responsible gene has not been identified. A linkage analysis of these disorders and single nucleotide polymorphisms (SNPs) in crossbreeds of the ataxic ICR line and the C57BL/6J strain revealed a SNP that was completely linked to the onset of these disorders. Therefore, we were able to predict the phenotype of crossbred infants by analyzing polymerase chain reaction (PCR) products including the SNP, before the phenotype emerged.

In this study, we examined histopathological changes in the spinal and trigeminal nerves and ganglia of genotypically homozygous Ataxic and wild-type infants of the crossbreed before the onset of disorders.

The homozygous (Ataxic) mice exhibited an ataxic gait on postnatal day (PD) 30, and many vacuoles accumulating neurofilaments (NFs) were found in the nerve fibers and ganglion cells of spinal nerves and the trigeminal nerve. On PDs, 13, 17, and 21 homozygous Ataxic mice appeared phenotypically normal, but NFs had accumulated in the vacuoles of nerves and ganglia. In the peripheral nerves on PD 9, small swellings containing accumulated NFs were found in nerve fibers but not in ganglion cells. No swellings or accumulations of NFs were found in the nerves of the homozygous Ataxic mice on PD 6, as in the wild-type mice of the same age.

These results suggest that the peripheral nerves develop normally but any impairment may appear in the growth or maturation of some neurons in the spinal nerves and the trigeminal nerve.

The impairment of homotetramerization of fructose-1,6-bisphosphatase owing to 2 Japanese founder mutations

We performed biochemical analyses of 2 candidate missense mutations (S164F and F194S) of the fructose-1,6-bisphosphatase gene (*FBP1*), found in a Japanese patient with episodic hypoglycemia and lactic acidosis.

Using the Flp-In system (Invitrogen/Life Technologies Corp., Carlsbad, CA, USA), we established 293 cell lines expressing the wild-type or the mutant FBP1. These cell lines had one copy of an FBP1-expressing construct at the same site of the genome. Therefore, each cell line might express wild-type or mutant FBP1 messenger (m) RNA in almost the same amounts. In fact, the expression of *FBP1* mRNA did not differ markedly among these cell lines. As opposed to the amount of mRNA, the amount of FBP1 protein was markedly decreased in the cell lines expressing mutant FBP1 than in those expressing wild-type FBP1. When the mutant FBP1-expressing cell lines were cultured with MG132, a proteasome inhibitor, the amount of mutant FBP1 protein by each increased in an MG132-dosage-dependent manner. Next, we examined whether the small amount of residual mutant FBP1 protein had enzymatic activity. An *in-vitro* assay for enzymatic activity revealed that the 2 mutant proteins had null activities. The formation of the homotetramer is necessary for the activity of FBP1. Using native polyacrylamide gel electrophoresis, we examined the multimerization of mutant FBP1 proteins. Thus, the two mutant FBP1 proteins, S164F and F194S, never formed homotetramers.

The 2 mutant FBP1 proteins S164F and F194S were previously and independently reported in Japanese cases of FBP1 deficiency. Our data provide new evidence that these 2 Japanese founder mutations impair the enzymatic activity of FBP1 by preventing the formation of homotetramers.

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

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

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

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

This study thus suggests that neural plate cells have a latent ability to be transformed into exterior epithelium of the neural plate, such as the neural crest, the PPE, and the embryonic ectoderm, through the action of BMP4 and fibroblast growth factor 2. The induced epithelium might be a precursor of all exterior epithelia of the neural plate. We are now investigating molecular cascades and cell differentiation in both the epithelium induced by the explant culture and the neural plate border in the embryo.

Establishment of a method for constructing a 3-dimensional gene expression model of inner-ear development

We attempted to establish a method for constructing 3-dimensional (3D) models of gene-expression patterns related to inner-ear development. To construct a 3D model, we prepared samples of whole mounts of in-situ hybridization, made cryosections, and captured images of each slice. The images were then imported into Amira (FEI Visualization Sciences Group, Bordeaux, France, and Zuse Institute Berlin, Berlin, Germany), a software program for 3D models, and reconstructed into a 3D model. From this model, we found differences in expression patterns between our data and previous reports. These differences were due to the intensity of gene expression, because our models detect expression at extremely low levels to allow detailed analysis. We also attempted to distinguish the intensity of gene expression with different colors, which indicated the center of the inducing area on the 3D models. Our 3D reconstruction models indicate gene expression in great detail.

Publications

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Department of Molecular Physiology

Shigeru Takemori, *Professor*

Maki Yamaguchi, *Assistant Professor*

General summary

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

Research Activity

Differential scanning calorimetry of water components in cells

Our previous studies of water in skeletal muscle sarcomeres found at least 5 water components distinguished by characteristic spin-spin relaxation rates. The overall interaction between water molecules and structural macromolecules has been shown to restrict water activity to differentiate these water components. However, details of the interaction remain unknown. To reveal the property of the interaction in these water components, we evaluated the phase-transition of water with differential scanning calorimetry, which gives direct information about interaction energies between molecules. As a first step, we observed the phase-transition process of pure water and confirmed that the pure water was supercool until 253 K and then froze reproducibly.

Dehydrating effects on contraction in skinned skeletal muscle

We recently found that small organic molecules, such as poly-ethylene-glycol (molecular weight, 8,350), which is thought to partially penetrate the sarcomere space, dehydrate skinned skeletal muscle similarly to the macromolecules that dehydrate osmotically. We investigated the contractile properties of skinned muscle dehydrated by macromolecules, such as Dextran T-500 (molecular weight, 500,000), or small organic molecules. Fibers dehydrated with Dextran showed increased maximum force and no change in Ca^{2+} -sensitivity. On the other hand, fibers dehydrated with polyethylene glycol showed significantly decreased maximum force and Ca^{2+} -sensitivity. This result suggests that the water groups removed from the sarcomere by polyethylene glycol differ from those removed by Dextran.

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

The viscoelastic properties of the myosin adsorbed to the surface of gold electrodes and its surrounding solution as a whole were studied with a molecular interaction analyzer (AFFINIX QN Pro, Initium, Inc., Tokyo).

When myosin was adsorbed at a density less than $0.2 \mu\text{g}/\text{cm}^2$, the viscoelastic change accompanying myosin adsorption was almost the same as the viscoelasticity of buffer without myosin. The resonance frequency falled with the weight of adsorbed myosin. This finding suggested that myosin adsorbed at a low density acts as a solid globular pro-

tein. On the other hand, when myosin was adsorbed at a higher density, a remarkable viscoelastic change was observed. Also, the binding of ATP to the myosin head changed the viscoelasticity of the protein. These results indicated that myosin acts as a protein having characteristic viscoelastic property.

Mutations of ryanodine receptor in malignant hyperthermia

Malignant hyperthermia (MH) is a pharmacogenetical complication of general anesthesia resulting from abnormal Ca^{2+} -induced Ca^{2+} release (CICR) via the type 1 ryanodine receptor (RyR1) in skeletal muscles. Although more than 200 mutations of the RyR1 gene have been reported in patients with MH, only a few of these mutations have been confirmed with experiments as being responsible for increases in CICR sensitivities, because complicated procedures are required to make the desired mutations in the long complementary (c) DNA of RyR1 and because of the low transfection efficiency of the mutant DNAs. We characterized the functional mutations of RyR1 in nonmuscle cells, specifically HEK293 cells with tetracycline-regulated RyR1 expression, prepared by improved method for making MH mutants in the cDNA of RyR1. Some mutations of the RyR1 were found to enhance CICR sensitivity; therefore, these mutations would be responsible for the incidence of MH. These results suggest that exploration of the functional mutations of RyR1 is effective for the preventive diagnosis of patients with MH disease.

Structural analysis of cardiac muscle fibers causing hypertrophic cardiomyopathy

The E244D- and K247R-troponin-T (TnT) mutants, which cause familial hypertrophic cardiomyopathy, have been shown to enhance calcium-dependent contraction on cardiac muscle fibers. To clarify the mechanism of this enhancement, we performed X-ray diffraction experiments with skinned muscle fibers to which wild-type/E244D/K247R mutant troponin T had been introduced. When E244D/K247R-TnT was introduced into the cardiac muscle fibers, intensity of the second actin layer line, which reflects structural change of tropomyosin on thin filaments, increased on contraction compared with that of fibers to which wild-type TnT had been introduced. This result indicates that in the E244D/K247R mutant, a larger shift of the tropomyosin would induce enhanced tension development and trigger the hypertrophy of cardiac muscle.

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Department of Cell Physiology

Susumu Minamisawa, *Professor*
 Norio Fukuda, *Associate Professor*

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

General Summary

The aim of research in our laboratory is to understand the regulatory mechanism of the cardiovascular system. In particular, we are interested in the mechanics of sarcomere contraction, Ca^{2+} homeostasis in the cardiac sarcoplasmic reticulum, the pathophysiology of cardiac fibrosis, and development of the cardiovascular system. In 2012, Professor Minamisawa succeeded Professor Emeritus Kurihara as the department chair. We established an experimental system to investigate small fetal arteries, such as the rat fetal ductus arteriosus. In addition, we developed an *in-vivo* nanoimaging system to observe sarcomere contraction in the ventricles of small animals, such as the rat and mouse.

Research Activities

Mechanism of sarcomere contraction in cardiac and skeletal muscles

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

We have demonstrated that the Frank-Starling mechanism is coordinately regulated in cardiac muscle via thin-filament “on-off” switching and titin-based changes in interfilament lattice spacing. In the present study, we investigated how the sarcomere length-dependence of active force production is altered in a knock-in mouse model of inherited dilated cardiomyopathy (DCM) with a deletion mutation ΔK210 in the cardiac troponin T gene. Confocal imaging revealed that the cardiomyocytes were significantly enlarged, especially in the longitudinal direction, in the hearts of ΔK210 knock-in mice, with striation patterns similar to those in wild-type hearts, suggesting that the number of sarcomeres is increased but that their length remains unaltered. To analyze the sarcomere length-dependence of active force, skinned muscles were prepared from the left ventricles of wild-type and ΔK210 mice. We found that the depressed Frank-Starling mechanism in the hearts of ΔK210 knock-in mice is the result of a reduction in thin-filament cooperative activation.

2. Real-time measurement of sarcomere length in the mouse heart *in vivo* by means of α -actinin-green fluorescent protein

Despite numerous studies performed under various experimental settings, the molecular mechanisms of contraction and relaxation of cardiomyocytes remain elusive *in vivo*. In the present study, we expressed green fluorescent protein (GFP) at sarcomeric Z-disks (α -actinin) by means of an adenovirus vector system in adult mice and performed real-time imaging of the movement of single sarcomeres in cardiomyocytes in the left ventricle under fluorescence microscopy at 10-nm precision (at 100 frames per second). We attempted to visualize single sarcomeres *in vivo* in open-chest mice under anesthe-

sia. We found that sarcomere length was 2.0 and 1.7 μm during diastole and systole, respectively, but varied by 0.3 μm even in the same left ventricular cell. We next found that sarcomere contraction occurred at the T-wave endpoint on electrocardiograms and was followed by an increase in left ventricular pressure. Finally, we successfully obtained Z-sectioning images ($Z = 1 \mu\text{m}$) of sarcomeres by means of a piezoelectric actuator and reconstructed the images to analyze changes in sarcomere length at nanometer precision during the cardiac cycle.

3. Real-time intracellular Ca^{2+} imaging in the heart

In the present study, we developed an experimental model for real-time imaging of intracellular Ca^{2+} in ventricular myocytes in the heart. Ca^{2+} waves were clearly observed at the cellular level in the isolated heart. Interestingly, randomly occurring Ca^{2+} waves or transients or both became synchronized by electric stimulation ($\sim 5 \text{ Hz}$). We also found that temperature control is highly important for intracellular Ca^{2+} imaging in the heart *in vivo*. Intracellular Ca^{2+} imaging in the heart will greatly enhance our understanding of the excitation-contraction coupling in health and disease.

4. Real-time nanoimaging of single sarcomere dynamics in rat neonatal cardiomyocytes via expression of actinin-*Aequorea coerulescens* GFP in Z-disks

A change in sarcomere length in cardiomyocytes causes a marked change in contractility. This intrinsic nature of sarcomere length-dependence of activation highlights the significance of simultaneous measurement of sarcomere length and intracellular Ca^{2+} concentration in localized areas of cardiomyocytes, at high spatial and temporal resolution. To directly visualize the motion of single sarcomeres at nanometer precision during excitation-contraction coupling, we applied cutting-edge nanoimaging technologies to primary cultured rat neonatal cardiomyocytes. We developed an experimental system for simultaneous nanoscale analysis of single-sarcomere dynamics and changes in intracellular Ca^{2+} concentration *in vivo* via expression of *Aequorea coerulescens* GFP in Z-disks. We first examined steady spontaneous sarcomeric oscillations at partial Ca^{2+} activation in primary-cultured rat neonatal cardiomyocytes. The present experimental system has a broad range of possible applications for unveiling single-sarcomere dynamics during excitation-contraction coupling in neonatal cardiomyocytes under various conditions.

Pathophysiological mechanisms of cardiac remodeling and fibrosis

Cardiac fibrosis is a maladaptive response to pathophysiological conditions, such as in cardiac hypertrophy and ischemic heart diseases. However, the effects of interstitial fibrosis on Ca^{2+} handling and contraction in myocardium remain unclear. We prepared pulmonary artery banding (PAB) rats as a model of cardiac hypertrophy. Four weeks after the operation, the right ventricular papillary muscles of PAB rats were dissected, and tension was measured with intracellular Ca^{2+} transients by using the photoprotein aequorin. On the basis of histological analysis, papillary muscles after PAB were clearly divided into 2 groups: an interstitial fibrosis group and a non-fibrosis with hypertrophy group. The peak Ca^{2+} in both interstitial fibrosis and non-fibrosis groups was significantly higher than that in the control group. However, peak tension in the interstitial fibrosis group was significantly less than that in the non-fibrosis and control groups. The

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

Regulation of cardiac sarcoplasmic reticulum ATPase activity

Impaired Ca^{2+} reuptake into the sarcoplasmic reticulum underlies a primary pathogenesis of heart failure in the aging heart. Sarcalumenin, a Ca^{2+} -binding glycoprotein located in the longitudinal sarcoplasmic reticulum, regulates Ca^{2+} reuptake by interacting with sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA). We found that the expression levels of both sarcalumenin and SERCA2 proteins were significantly downregulated with age and that the downregulation of sarcalumenin protein preceded that of SERCA2 protein. Using senescent sarcalumenin knockout mice, we found that sarcalumenin plays a critical role in maintaining Ca^{2+} transport activity of SERCA2a and cardiac function in the senescent population.

Development and pathogenesis of the great arteries

Molecular mechanism of closure of the ductus arteriosus

The ductus arteriosus is a mysterious and interesting artery. The ductus arteriosus is an essential vascular shunt between the aortic arch and the pulmonary trunk during fetal development. The ductus arteriosus closes immediately after birth in accordance with its smooth muscle contraction and vascular remodelling. When the ductus arteriosus fails to close after birth, the condition is known as patent ductus arteriosus (PDA), which is a common problem in premature infants. Although cyclooxygenase inhibitors are often used to treat PDA, their efficacy is often limited. Because thromboxane A_2 (TXA_2) induces vascular contraction via the TXA_2 receptor, we hypothesized that TXA_2 receptor stimulation promotes ductus arteriosus closure. The selective TXA_2 receptor agonists U46619 and I-BOP caused constriction of the fetal ductus arteriosus in a dose-dependent manner on embryonic days 19 and 21. In addition, U46619 exerted a vasoconstrictive effect in 2 different postnatal PDA models: premature PDA and hypoxia-induced PDA. Furthermore, we found that U46619 at lower concentrations (up to 0.05 mg/g of body weight) had a minimal vasoconstrictive effect on other vessels and did not induce microthrombosis in the pulmonary capillary arteries. Therefore, we conclude that low-dose TXA_2 receptor stimulation constricts the ductus arteriosus with minimal adverse effects, at least in rat neonates, and our results could lead to an alternative potent vasoconstrictor for PDA.

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Department of Biochemistry

Kiyotsugu Yoshida, *Professor*
Tadashi Asakura, *Associate Professor*

Koji Takada, *Associate Professor*

General Summary

Tumor is a genetic disease. The fundamental defect of tumor cells is a deregulated proliferation that results from the progressive accumulation of genetic and epigenetic alterations. These alterations invariably affect the regulatory pathways that govern the proper cellular responses to these myriad signals. Normal proliferative cells are endowed with the ability to choose from among growth, quiescence, differentiation, and apoptosis. The execution of these alternative choices is influenced by physiological factors and stress to achieve a controlled and balanced proliferation. Our research is directed at elucidating signaling pathways that allow normal cells to distinguish from among proliferation, differentiation, and apoptosis.

Research Activities

Cancer research

1. Dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 phosphorylation of c-Jun/c-Myc controls tumor progression by monitoring the G1/S transition

Transcription factors c-Jun and c-Myc are indispensable regulators of the G1/S transition, and their expression is tightly regulated at transcriptional and posttranslational levels. Dysregulation of this expression leads to tumor development and progression. Degradation of c-Jun/c-Myc is triggered by sequential phosphorylation by unknown priming kinase(s) and glycogen synthase kinase 3 beta. This year, we clarified that dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 (DYRK2) functions as a priming kinase for c-Jun/c-Myc whose phosphorylation is required for subsequent glycogen synthase kinase 3 beta phosphorylation and proteasomal degradation. Stable knockdown of DYRK2 results in aberrant expression of c-Jun and c-Myc and facilitates cell proliferation and tumor progression *in vivo*. Furthermore, DYRK2 expression is down-regulated in invasive breast cancer tissues. Notably, expression of c-Jun and c-Myc is elevated in tissues with low DYRK2 expression. Taken together, these results suggest that depletion of DYRK2 expression contributes to tumorigenesis.

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

E-Cadherin was suppressed via expression of transcriptional repressor gene *ZEB1* in endometrial carcinoma Ishikawa cells resistant to the proteasome inhibitor epoxomicin. This finding suggests that the expression of *ZEB1* is involved in the suppression of dual-specificity protein phosphatase 6.

3. Targeted chemotherapy with polymeric micelles against CD147-expressing carcinoma cells

Because a specific accumulation of cytotoxicity was observed in CD147-expressing cells treated with glutathione-doxorubicin conjugate-encapsulated anti-CD147 antibody-labeled micelles, we prepared tumor-bearing mice for *in vivo* investigation of the chemotherapeutic effect.

Other research

1. Mechanistic elucidation of cadmium-induced cytotoxicity

We analyzed the cadmium-induced cytotoxicity with proximal tubular HK-2 cells by using reagents that modulate the selective proteolytic system. The results strongly suggest that the ubiquitin-proteasome system is responsible for resistance to this toxicity.

2. Production of fibrinogen by a well-differentiated human hepatoma cell line

We developed an efficient and economical system for fibrinogen production by optimization of the FLC-7 cell culture system comprising 2 serum-free media (ASF104N and IS-RPMI) and a radial-flow bioreactor. We expect this system will be used to develop methods of risk-free fibrinogen preparation.

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

Ubiquitin-specific proteases (USPs) are deubiquitinating enzymes that remove ubiquitin from specific protein substrates and modulate the ubiquitin-proteasome system. Recently, *USP46* was identified as a quantitative trait gene responsible for decreasing immobility time in the tail suspension test of the CS mouse. The CS mouse has a 3-bp deletion coding for Lys 92 of the protein USP46, but the effect of the deletion mutation on deubiquitinating enzyme activity is not clear. To construct a measurement system for wild-type and mutant USP46 activity, we prepared an expression system using an episomal vector in mammalian cells. An episomal vector bearing USP46 complementary DNA was transfected into HeLa cells and stably expressed the proteins (wild-type and mutant). However, the USP46 levels in the transfected HeLa cells were lower than in nontransfected HEK293 cells. Hence, we assumed that USP46 forms deubiquitinating enzyme complexes with partner proteins and that USP46 protein is unstable by itself. A search for known proteins interacting with USP46 identified a WD-domain repeat (WDR) 20, which is a protein containing WD40-repeat motifs and a subunit for USP12 deubiquitinating enzyme complexes, as stabilizing factor for USP46 proteins.

4. Primary structural analysis of host-defense peptides of *Xenopus*

We determined amino acid sequences of host-defense peptides separated from skin secretions of several species of *Xenopus* and elucidated the primary structures of these peptide groups.

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Department of Molecular Biology

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

Akihiro Oguro, *Assistant Professor*

General Summary

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

Research Activities

Role of AZ2 in c-MYC degradation

We have previously found in cultured cells that AZ2 accelerates c-MYC degradation by the proteasome in an ubiquitin-independent manner. When investigating the roles of AZ2-mediated c-MYC degradation, we noticed that hypoxia and undernutrition have been reported to cause downregulation of c-MYC. Knockdown of AZ2 under these conditions inhibited downregulation of c-MYC and increased the frameshift efficiency of AZ2. Thus, our results suggest a novel pathway of hypoxia-induced c-MYC degradation mediated by polyamines and AZ2 in an ubiquitin-independent manner. c-MYC has been found in both the nucleolus and the nucleus. Proteasome inhibition leads to c-MYC accumulation within the nucleolus. Interestingly, AZ2 also localized to the nucleolus in the presence of proteasome inhibitor. Furthermore, when c-MYC and AZ2 were coexpressed in the cells, their nucleolar localizations were identical. These results suggest that AZ2 regulates c-MYC in the nucleolus.

Fluorescent visualization of cancer cells by monitoring of cellular polyamines

We are developing a method to visualize cancer cells by combining the polyamine-dependent frameshift mechanism of AZ, an endogenous cellular polyamine sensor, and fluorescent protein techniques. Last year a reporter, constructed from the entire protein-coding region of human AZ1 messenger (m) RNA and the enhanced green fluorescent protein (EGFP) gene that is inserted immediately downstream of the pseudoknot structure, showed increases in both EGFP fluorescence and the frameshift product in response to the addition of polyamines to the culture medium. This year, we further improved this construct by inserting the ECFP gene immediately in front of AZ1 mRNA as a nonframeshifting control and by inserting the Keima-Red gene immediately downstream

of the pseudoknot structure as a frameshifting confirmation. However, the construct did not respond to the addition of polyamines to the culture medium. Reconsideration of fluorescent reporter genes and their insertion positions is needed to increase the polyamine response.

Characterization of hematopoietic stem cells in the livers of fetal AZ1 knockout mice

We have previously shown that knockout of the AZ1 gene increases tissue polyamine levels, decreases the number of multipotent hematopoietic progenitor cells (MPPs) in fetal liver, and results in severe anemia and embryonic death. Because asymmetric division of hematopoietic stem cells (HSCs) generates MPPs, we used a long-term competitive bone-marrow repopulating assay to evaluate the hematopoietic ability of HSCs in the livers of *AZ1*^{-/-} fetal mice. Consequently, we found that the number of repopulating units/HSC was lower in the livers of *AZ1*^{-/-} fetal mice than in those of wild-type mice. This result possibly explains the decrease in MPPs in the livers of *AZ1*^{-/-} fetal mice.

Multiple forms of mouse Azin1 mRNA are differentially regulated by polyamines

Azin1, a positive regulator of cellular polyamines, is induced by various proliferative stimuli and is repressed by polyamines. We have found multiple forms of *Azin1* transcript formed by alternative splicing and initiation of transcription from putative alternative start sites. This year, we first showed that a novel splice variant, *Azin1*-X, with a premature termination codon encoding a C-terminal truncated form of protein, is subject to nonsense-mediated mRNA decay. Next, 2-difluoromethylornithine, an inhibitor of polyamine synthesis, increased both transcription from the canonical transcription start site and the ratio of the full-length mRNA to *Azin1*-X mRNA; in contrast, polyamines showed the opposite effects. Thus, polyamines regulate 2 novel steps of *Azin1* expression, namely transcription and a particular splicing pattern, both of which may affect the level of mRNA encoding the full-length active Azin1 protein.

Polyamine-related research with stable isotopes

We have identified ATP citrate lyase (ACLY) as a candidate AZ2-interacting protein. We found that nuclear localization of ACLY was increased in the presence of AZ2, although localization of ACLY is predominantly cytoplasmic when expressed alone. We are establishing assay systems for ACLY activity and histone acetylation which employ a mass spectrometer with a stable isotope-labeled citrate (¹³C) to reveal the effect of AZ2 on ACLY activity. We are also attempting to determine polyamine flux by means of liquid chromatography/tandem mass spectrometry using stable isotope-labeled ornithine as a metabolic substrate of polyamines to clarify the effect of an Azin1 variant on polyamine levels in the cell. To eliminate the effect of impurities in samples, we are also establishing a liquid chromatography/tandem mass spectrometry detection method using stable isotope-labeled polyamines as internal standards for absolute quantification.

Isolation and analyses of polyamine-binding RNA aptamers

RNA aptamers have the potential applications for both clinical and research. In

particular, aptamers are useful for exploring RNA-binding sequences and structures for target molecules. We are revealing general polyamine-binding RNA sequences and structures by analyzing polyamine-binding sites on isolated RNA aptamers. A part of a previously isolated antispermine aptamer consisting of a stem-loop with bulge-out structure has affinity for spermine. We found that a bulge region with 4 neighboring base pairs possessed a lower affinity for spermine. Moreover, the other stem region also possessed a weak binding activity. Interestingly, the existence of both regions is important for the strong binding activity. Some base replacements in one region abolished the binding activity of the other region. This finding indicates that these 2 regions work together to achieve the full binding activity.

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

Amplification of chromosome 17q21-24 has frequently been observed in ovarian clear cell carcinoma (CCC). However, the driver gene of the region has not been identified. Aberrant expression of microRNAs has been shown to be involved in oncogenesis. MicroRNA-21 (miR-21), encoded on 17q21-24, is a frequently overexpressed miRNA in many types of cancer. Analysis of clinical samples revealed overexpression of miR-21 and repression of phosphatase and tensin homologue (PTEN) in cases of CCC with amplification of 17q21-q24. We profiled *miR-21*, *PTEN* mRNA, and PTEN protein expression in 5 CCC cell lines. We selected RMG-II for function analysis because it was the CCC cell line that had the most prominent miR-21 overexpression and lowest PTEN protein expression. Knockdown of miR-21 in RMG-II cells increased *PTEN* mRNA and protein expression and cell viability but did not change invasive ability. We identified *PTEN* as a target gene for miR-21 using a dual luciferase reporter assay. Our study revealed that miR-21 is a possible driver gene other than *PPM1D* for 17q21-24 amplification in CCC.

Department of Pharmacology

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

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

General Summary

The research interests of the Department of Pharmacology include:

- 1) Synaptic transmission and its modulation in the basal ganglia and basal forebrain (Toshihiko Momiyama)
- 2) Neural control of breathing in aquatic vertebrates (Naofumi Kimura)
- 3) Intracellular function of endozepine (Yuji Ohno)
- 4) Study of glucocorticoid secretion by activation of purinergic receptors in a human-derived adrenocortical cell line (Haruhisa Nishi)
- 5) Firing patterns of neurons in the pontine nuclei (Taro Ishikawa)
- 6) The basic mechanism of a ketogenic diet: a purinergic autocrine regulation of CA3 pyramidal neurons (Masahito Kawamura)
- 7) Synchronization of periodic activities in cerebrocerebellar interaction (Misa Shimuta)

Research Activities

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

Electrophysiological studies using slice patch-clamp recording were performed to analyze synaptic transmission; its modulation by neuromodulators, such as dopamine and serotonin, and their developmental changes in the nigrostriatal or mesolimbic dopaminergic system and in the cholinergic system of the basal forebrain. These systems are involved in various psychological functions as well as their disorders, including Parkinson's disease and Alzheimer's disease. Electrochemical analyses were also performed with a new biosensor material, carbon-nanotube, to elucidate the mechanisms of catecholamine release in the midbrain.

Another issue is the regeneration of synapses and local circuits after basal-ganglia-related disorders. Electrophysiological, morphological, and behavioral studies were performed to elucidate the mechanisms and time course of the reconstruction of synaptic organization and transmission and the functions of whole animals in Parkinson's disease model rats or cerebral ischemia model rats. In addition, the role of the phosphatidylinositol system in basal ganglia synaptic transmission was analyzed.

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

Neural control of breathing in aquatic vertebrates

Yawning in mammals has been considered a respiration-related behavior because of

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

Intracellular function of endozepine

In the central nervous system, endozepine is an endogenous anxiogenic peptide that is able to suppress the binding of gamma aminobutyric acid (GABA) to GABA_A receptors through the association of the peptide with a benzodiazepine receptor. In addition to endozepine having this extracellular function, we have suggested it has an intracellular function in bovine adrenocortical cells. To express the peptide in *Escherichia coli*, we extracted messenger (m) RNA from bovine adrenocortical cells, and the complementary DNA of endozepine was amplified for insertion into expression vectors. The peptide expressed by *E. coli* could not exert sufficient function. In view of posttranslational modification, including glycosylation, endozepine should be expressed by mammalian cells, such as HEK 293. We would like to examine the posttranslational modifications and intracellular functions of endozepine expressed by mammalian cells.

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

H295R, a human adrenocortical cell line, was used to investigate purinergic system function, especially P2Y₁ purinergic receptors (P2Y₁R), in human adrenocortical glucocorticoid secretion. Protein levels first confirmed the successful knockdown of P2Y₁R mRNA with the short hairpin RNA method in H295R. Glucocorticoid secretion induced by 2-methylthio-ATP, a P2Y₁R-selective agonist, was significantly reduced in the knockdown cells. This reduction demonstrates the involvement of P2Y₁R in glucocorticoid secretion. To further examine the nucleotide breakdown responsible for the inactivity of the endogenous purine agonists, their metabolites were tested in H295R. Changes in both mRNA expression of ecto-nucleotidase and protein were observed. Furthermore, both luciferin-luciferase and radioisotope techniques indicated a nonrapid metabolism of the purine, sufficient to activate purinergic receptors resulting in glucocorticoid secretion. These findings indicate that P2Y₁R activation contributes to the glucocorticoid secretion in H295R.

Firing patterns of neurons in the pontine nuclei

Sensory and motor signals from the cerebral cortex are delivered to the cerebellum via the pontine nuclei in the brainstem. To elucidate the synaptic transmission and the firing patterns of the neurons in these relay nuclei, we performed whole-cell patch-

clamp recordings from the principal neurons in the pontine nuclei. These novel experiments provided evidence that the pontine neurons receive bursting synaptic inputs at the frequency of the theta band (4 to 5 Hz) and that they fire action potentials at this oscillatory frequency.

The basic mechanism of a ketogenic diet: a purinergic autocrine regulation of CA3 pyramidal neurons

A ketogenic (low-carbohydrate, high-fat) diet has been successfully used to treat pediatric and medically refractory epilepsy. The mechanisms underlying the success of ketogenic diet therapy, however, are poorly understood. A ketogenic diet reportedly increases ATP concentrations in the central nervous system and causes mild hypoglycemia. To elucidate the role of extracellular purines underlying the anticonvulsant effect of the ketogenic diet, whole-cell voltage-clamp recordings were made from CA3 pyramidal neurons in acute hippocampal slices of rats. Under conditions of reduced extracellular glucose and high intracellular ATP concentrations, CA3 pyramidal neurons hyperpolarize themselves via direct ATP release through pannexin-1 channels with the subsequent activation of adenosine A₁ receptors. This autocrine regulation might be an important mechanism underlying the success of a ketogenic diet.

Synchronization of periodic activities in cerebrocerebellar interaction

Neural signals from the cerebral cortex reach cerebellar granule cells. However, this signaling pathway has not been investigated with intracellular recordings from the cerebellar granule cells. Thus, we simultaneously recorded field potentials from the cerebral sensory cortex and the whole-cell current from single cerebellar granule cells in anesthetized rats. These experiments showed that the activity of the cerebral cortex is synchronous to the synaptic inputs in the cerebellar granule cells and that such synchronous activities are oscillatory at the theta frequency.

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Department of Pathology

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

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

General Summary

The aim of our research in the Department of Pathology is to elucidate, on the basis of morphology, pathogenesis and morphological changes. The materials used are human specimens from autopsies, surgical resections, and biopsies. These materials are examined with light microscopy, electron microscopy, morphometry, immunohistochemistry and molecular pathology.

Research Activities

Pathology of liver

1. Pathology of nonalcoholic steatohepatitis

We performed studies until the last fiscal year on how tissue injury in the precirrhotic stage of nonalcoholic steatohepatitis (NASH) changes over time. This research involved 3-dimensional histological reconstruction of the liver using serial sections and focused on 3-dimensional changes in liver fibrosis and the course of blood vessels. We found progression of fibrosis around the central veins, with connections to fibrosis around adjacent central veins, thus leading to fibrous bridging. Extension of these changes throughout the liver resulted in the formation of fibrosis surrounding the portal tracts. However, even at this stage, the fibrous bridging between the central veins and portal tracts was limited to a few sites. The reason fibrous bridging was limited was that the portal vascular structure was preserved, with little injury involving the portal tracts or parenchyma. Over time, arteries developed in fibrotic areas around the central vein, but these arteries clearly arose from arteries in the portal tracts.

The incidence of ballooning degeneration of hepatocytes in NASH varied among individual cases. Ballooning degeneration was prominent in central areas in the hepatic lobule and parenchymal margins surrounding fibrotic lesions and in areas of fibrous bridging between the central areas in the hepatic lobules and portal tracts. These findings suggest that progression of fibrosis in NASH is associated with ballooning degeneration as well as with inflammation. Considering that inflammatory changes are relatively mild and sporadic in NASH, we speculate that the ballooning degeneration that occurs before fibrosis is important in the mechanism of fibrosis.

2. Findings in the background liver in non-B, non-C hepatocellular carcinoma

This study focused on past medical history and the histopathological changes of the liver in areas other than hepatocellular carcinoma (HCC) in 36 patients with non-B, non-C

HCC. Excessive alcohol use, NASH, and lifestyle-related diseases, such as obesity and diabetes, were thought to be involved. However, histopathological examination found that some livers had extensive remodeling, as seen in hepatic fibrosis and cirrhosis; some livers had mild, nonspecific damage; and other livers had an almost normal appearance. In other words, the background liver lesions varied considerably.

3. Expression of prominin 1 (CD133) protein in HCC genesis and metastasis

The protein prominin 1 is constitutively expressed in normal, nonneoplastic hepatocytes in human fetuses and adults. The expression of prominin 1 protein in the normal liver is attenuated or lost during the malignant transformation of hepatocytes. Loss of the intrinsic physiological function of prominin 1 is thought to be associated with a high likelihood of HCC. However, in the process of HCC metastasis, no substantial changes in prominin 1 expression were observed. Therefore, there is probably no direct causal association between prominin 1 and cancer metastasis.

4. The origin of proliferative ductules in obstructive jaundice

In early obstructive jaundice (up to 14 days after onset), bile ductules appear near the portal tracts and are intermingled with hepatic cords. In some cases, these bile ductules are also intermingled with preexisting interlobular bile ducts. In late obstructive jaundice (more than 14 days after onset), these bile ductules continue to proliferate, fibrosis occurs, the older bile ductules are surrounded by fibrosis, and there is a morphologically similar appearance to preexisting interlobular bile ducts.

On the basis of these morphological findings, we performed immunostaining for bile ductule CK7 and a hepatocyte marker. The bile ductules were positive for CK7, and the hepatocytes were positive for the hepatocyte marker. In obstructive jaundice, hepatocytes with coexpression of CK7 and the hepatocyte marker increased over time. Later, hepatic cords that also contained stem cells expressing only the hepatocyte marker were prominent, and in some areas, there was contiguity between the hepatic cords and proliferative ductules. This finding suggests a close association among these 3 structures. We conclude that bile ductules are formed from hepatocytes via hepatocytes that coexpress both CK7 and hepatocyte markers.

Gastrointestinal pathology

1. Risk factors for metastasis from small cancers of the colon to lymph nodes were examined in 203 submucosal invasive carcinomas and 62 advanced carcinomas 20 mm or less in diameter. For submucosal invasive carcinoma, 6 risk factors (macroscopic depression, intramucosal growth, submucosal invasiveness, invasive histologic type, budding, and vessel invasion) were established, and multivariate analysis was performed. In particular, vessel invasion was accurately evaluated with immunostaining for D2-40 and CD31 and Elastica van Gieson staining. The most important risk factor for metastasis in submucosal invasive carcinoma was vessel invasion, as evaluated with D2-40, CD31 and Elastica van Gieson staining.

Urological pathology

1. Renal pathology

We analyzed the morphology of renal tissue. Postmortem tissue specimens were

examined from the kidneys of 43 patients without known lesions. Renal growth and changes with aging were evaluated on the basis of arterial size, arterial wall thickness, and morphological changes in the glomeruli. We found that inner and outer diameters of arteries increased with age and that the glomerular capillaries and mesangial cells increased with age after the age of 10 years. We also found that in patients with high kidney weight, the numbers of morphologically preserved glomeruli and renal tubules per unit area were smaller.

2. Renal cell carcinoma

We evaluated cases of cystic renal cell carcinoma (RCC). There was a morphological spectrum ranging from tumors being entirely cystic to being partially cystic. In particular, when the cystic area was 50% or more, there were no metastases, and the prognosis was good. Among 25 patients with bilateral RCC, 7 patients had metastases from the contralateral kidney, and 8 patients had lesions that had clearly developed separately.

3. Prostate cancer

The relationship between pAKT and ETS-related gene (ERG) expression in clinical carcinoma was evaluated. pAKT was expressed in about half of Japanese men with prostate cancer, and ERG was expressed in about 25%. There was a negative correlation between the expression frequency of pAKT and that of ERG. This finding suggests that these 2 pathways of malignant transformation are independent. In addition, the frequency of ERG expression in Japanese men was only about half that in Western men and suggests ethnic differences in the malignant transformation pathway. In a study of latent prostate carcinoma, such data as the incidence of latent carcinoma, cancer volume, and age in recent autopsy cases were compared with previously accumulated latent carcinoma data (from the 1980s). This comparison showed a doubling in the incidence of cancer and a particular increase in larger tumors.

Gynecological pathology

1. We examined the histopathology of endometrioid adenocarcinoma treated with hormone therapy (medroxyprogesterone acetate) to preserve fertility (maintain the ability to become pregnant). We found decreased cellular atypia, signs of glandular secretion, and decidual tissues in the stroma. However, the basic pattern of glandular proliferation showed few changes. Live births were achieved in a small number of cases. However, no histological diagnostic criteria for cancer after hormone therapy or guidelines concerning when to discontinue treatment have been established. These are issues for further investigation.

2. Diagnostic significance and histogenic significance of TSSC3 and p57 staining in trophoblastic disease.

Breast pathology

1. We reviewed 191 breast lesions that were on the borderline between benignancy and malignancy. There was intraobserver disagreement about the diagnosis in some patients and a discrepancy in diagnosis ranging from benign (hyperplasia) to malignant (ductal carcinoma in situ). In particular, there were strong tendencies for discrepancies in

diagnosis among cases in which myoepithelium was present only in the lumen borders of intraductal lesions.

2. Comparison between immunostaining results and fluorescence in-situ hybridization analysis for human epidermal growth factor receptor 2 testing in breast cancer.

Other pathology

1. We evaluated dermokine expression in tumor lesions derived from oral stratified squamous epithelium. We found that dermokine expression differed between normal oral mucosa and the skin and that expression in tumor lesions was closely related to tumor differentiation.

2. We reported pathological findings in an autopsy case of Chagas disease.

3. We examined systemic changes due to graft-versus-host disease after bone marrow transplantation.

Publications

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Department of Virology

Kazuhiro Kondo, *Professor*

General Summary

Human herpesviruses (HHVs) are capable of establishing lifelong latent infections of their hosts and are reactivated frequently. We are studying the molecular mechanism of latency and the pathogenesis of HHV-6 and searching for latent proteins of HHV-6 associated with chronic fatigue syndrome (CFS) and mood disorders. Additionally we are attempting to apply HHV-6 and HHV-7 as tools to study the mechanism of fatigue and as viral vectors for gene therapy. Furthermore, we studied the relationship between herpes simplex virus (HSV) reactivation and Alzheimer's disease (AD).

Research Activities

Increase in the immunoglobulin G avidity index due to HSV type 1 reactivation and its relationship with cognitive function in amnesic mild cognitive impairment and AD

After infection with HSV type 1 (HSV-1), latent infection persists for life in the trigeminal ganglion, and reactivation results in an outbreak of cold sores around the mouth. Many previous studies have reported HSV-1 reactivation to be a risk factor for AD. This study enrolled subjects with AD (n = 85); subjects with amnesic mild cognitive impairment (aMCI), a prodromal stage of AD (n = 34); and healthy control subjects (n = 28). The avidity index of anti-HSV-1 immunoglobulin (Ig) G antibodies—a known indicator of HSV-1 reactivation—was measured to clarify the relationship between HSV-1 reactivation and symptoms of cognitive function in AD.

Cognitive function in AD and aMCI was evaluated with scores from the Mini-Mental State Examination and the Frontal Assessment Battery. The results showed that the subjects with aMCI, in whom cerebral function is better preserved than in subjects with AD, had a higher anti-HSV-1 IgG antibody avidity index than did subjects with AD or healthy controls. Furthermore, the anti-HSV-1 IgG antibody avidity index was even higher in the subjects with high Mini-Mental State Examination scores on orientation to time and 3-step command subscores. We observed a negative correlation between the anti-HSV-1 IgG antibody avidity index and the plasma concentration of brain-derived neurotrophic factor, which is an indicator of encephalitis. This finding suggests that HSV-1 reactivation, as observed through an increase in the anti-HSV-1 IgG avidity index, does not progress to encephalitis.

These results suggest that HSV-1 reactivation occurs from the stage of aMCI, which is prodromal to AD, and can affect AD symptoms without an intermediary stage of severe encephalitis. The study suggests that the anti-HSV-1 IgG antibody avidity index is a useful biomarker for the early diagnosis of aMCI or AD and that antiviral medication to treat HSV-1 could play a role in preventing the onset of AD.

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

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

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

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

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

Novel gene therapy viral vector using nononcogenic lymphotropic HHV

Gene introduction into T cells is a useful technique for gene therapy of human immunodeficiency virus (HIV) infection and the immunotherapy of fatal diseases, including cancer. This method now relies on vectors derived from members of the lentivirus family of retroviruses to introduce genes into T cells. A major advantage of retroviral vectors is the high efficiency with which they introduce genes into target cells. However, the pathogenicity of the native virus has long caused unease regarding the use of viral vectors. In particular, oncogenicity is a characteristic of wild-type retroviruses; another risk factor is the potential recombination of retroviral vectors with endogenous retroviruses in the host to yield replication-competent virus.

Despite the use of retroviral vectors, efficiently introducing target genes into immunocytes, such as T cells, is difficult. In addition, retroviral vectors carry risks associated with the oncogenicity of the native virus and the potential for introducing malignancy in recipients due to genetic carryover from immortalized cells used during vector production. To address these issues, we have established a new virus vector that is based on HHV-6, a nononcogenic lymphotropic HHV that infects CD4⁺ T cells,

macrophages, and dendritic cells. In the present study, we altered the cell specificity of the resulting recombinant HHV-6 by knocking out the U2-U8 genes. The resulting virus proliferated only in activated umbilical cord blood cells but not in peripheral blood cells. Umbilical cord blood cells produced replication-defective recombinant virus in a sufficiently high titer to make unnecessary the use of immortalized cells during vector production. The HHV-6 vectors led to high rates (>90%) of gene transduction in both CD4+ and CD8+ T cells. These viruses showed low-level replication of viral DNA that supported expression of the induced genes that was greater than that of other methods but was insufficient to support the production of replication-competent virus. Furthermore, HHV-6 vectors containing short hairpin RNAs against CD4 and HIV Gag markedly inhibited the production of these proteins and of HIV particles. Our results demonstrate the utility of HHV-6 as a new noncarcinogenic viral vector for treating immunologic diseases and for immunotherapy.

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

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

We have studied the expression of SITH-1 by examining the prevalence of anti-SITH-1 antibodies in persons with CFS or mood disorders and in healthy persons. Antibody detection was by indirect immunofluorescence and enzyme-linked immunosorbent assay. Next, an open reading frame of SITH-1 was linked downstream of a glial fibrillary acidic protein promoter and expressed in glial cells of mice using an adenovirus vector. After growth, the mice were analyzed with the tail suspension test, prepulse inhibition, and locomotor activity.

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

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Department of Bacteriology

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

Akiko Tajima, *Assistant Professor*
Shinya Sugimoto, *Assistant Professor*

General Summary

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

Research Activities

Purification, crystallization, and preliminary X-ray diffraction analysis of the S. epidermidis extracellular serine protease Esp

Esp, an extracellular serine protease from *S. epidermidis*, has been shown to inhibit *S. aureus* biofilm formation and nasal colonization. The full-length 27-kDa pro-Esp was purified and digested with thermolysin to obtain mature Esp. The mature Esp containing 216 residues crystallized in space group P2(1), with unit-cell parameters $a = 39.5$, $b = 61.2$, $c = 42.5$ Å, $\beta = 98.2^\circ$, and 1 molecule in the asymmetric unit, with an estimated solvent content of 42%. A diffraction data set has been collected to 1.8-Å resolution on a rotating-anode home-source facility.

Excreted molecular chaperones ClpB and DnaK stimulate bacterial biofilm formation

We have recently identified molecular chaperones ClpB and DnaK in the biofilm matrix fraction of *S. aureus*. Here, we show that extracellular ClpB and DnaK play important roles in the quality control of bacterial biofilms. Cytological analyses showed that ClpB and DnaK were attached to the cell surface of *S. aureus* embedded in biofilms during all phases of biofilm formation. Interestingly, extracellularly supplemented ClpB and DnaK stimulated *S. aureus* biofilm formation in a dose-dependent manner, whereas GroEL did not. Mutational analyses indicated that the hexamer formation and the disaggregating chaperone activity of ClpB are dispensable for biofilm promotion and, at least, the N, D1, and M domains are necessary for this extracellular function. ClpB may act as a “molecular glue” to stimulate cell-cell interaction or cell-surface interaction or both.

Spatiotemporal dynamics of the multifunctional biofilm matrix protein Eap

We have identified extracellular adherence protein (Eap), a multifunctional biofilm matrix protein related to various infectious diseases, from an *S. aureus* robust biofilm former. *In vitro* experiments revealed that purified Eap promoted biofilm development by *S. aureus*. We are now characterizing *eap*-knockout mutants to understand the

functions of Eap in biofilm development and virulence. Furthermore, we are analyzing the spatiotemporal dynamics of this protein within biofilms.

Observation of biofilms by atmospheric scanning electron microscopy

We directly observed biofilms formed by various bacteria in buffer with the newly developed atmospheric scanning electron microscope. This microscope features an open sample dish with a pressure-resistant thin film window in its base, through which the scanning electron microscopy beam scans samples in solution, from below. The locations of biofilm matrix components (proteins and polysaccharides) and fine structures of extracellular membrane vesicles and nanotubes were visualized using immunolabeling and optimized staining methods.

Effects of bacteriocins on MRSA biofilm

Control of biofilms formed by microbial pathogens is an important subject for medical researchers, because the development of biofilms on foreign-body surfaces often causes biofilm-associated infections in patients with indwelling medical devices. The present study examined the effects of different kinds of bacteriocin, which are ribosomally synthesized antimicrobial peptides produced by certain bacteria, on biofilms formed by a clinical isolate of MRSA. The activities and modes of action of 3 bacteriocins with different structures (nisin A, lacticin Q, and nukacin ISK-1) were evaluated. Vancomycin, a glycopeptide antibiotic used in the treatment of MRSA infections, showed bactericidal activity against planktonic cells but not against biofilm cells. Among the tested bacteriocins, nisin A showed the highest bactericidal activity against both planktonic cells and biofilm cells. Lacticin Q also showed bactericidal activity against both planktonic cells and biofilm cells, but its activity against biofilm cells was significantly lower than that of nisin A. Nukacin ISK-1 showed bacteriostatic activity against planktonic cells but not against biofilm cells. Mode-of-action studies indicated that pore formation leading to ATP efflux is important for the bactericidal activity against biofilm cells. Our results suggest that bacteriocins that form stable pores on biofilm cells would be highly effective for the treatment of MRSA biofilm infections.

High-throughput screening of antibiofilm compounds

A potential strategy for preventing and treating biofilm-associated infections is to use small molecules that inhibit biofilm development. We are now performing high-throughput screening (HTS) to identify compounds that inhibit bacterial biofilm development; we are collaborating with the University of Tokyo, which has a chemically diverse small-molecule library (200,000 compounds). We have established a crystal violet staining assay of biofilm that is suitable for HTS. Additionally, we have designed a screening robot system that automates the dispensing of compounds to assay plates, cell-culture handling, and activity measurement. Screening studies with several bacterial strains that can form biofilm are now in progress to assay 10,000 different conditions each month. So far, several compounds with inhibitory activity against *S. aureus* biofilm have been obtained. Hereafter, we will perform large-scale HTS to find more effective compounds, and then we will analyze in detail the composition of the

molecular interactions of these compounds with the bacteria.

Pathogenicity of E. coli O157 entering the VNC state

Some *E. coli* O157 strains become VNC under environmental stress conditions and evade detection with conventional culture methods. We showed that the addition of catalase to the culture medium resuscitated O157 from the VNC state to a culturable state and that decreased sigma factor S activity (encoded by the *rpoS* gene) caused bacteria to enter the VNC state. To investigate the pathogenicity of VNC cells, we infected germ-free mice with VNC O157 strains and found that the resuscitated VNC cells colonized the gut and induced death in all mice.

Mechanism of bacterial ATP secretion

ATP modulates immune cell functions, and ATP derived from gut commensal bacteria promotes the differentiation of T helper 17 (Th17) cells in the intestinal lamina propria. We recently reported that *Enterococcus gallinarum*, isolated from mice and humans, secretes ATP. We have since found and characterized several ATP-secreting bacteria. Of the tested enterococci, *Enterococcus mundtii* secreted the greatest amount of ATP ($>2 \mu\text{M}/10^8$ cells) after overnight culture. Glucose was essential for ATP secretion from *E. mundtii*. Analyses of energy-deprived cells demonstrated that glycolysis is the most important pathway for bacterial ATP secretion. Furthermore, exponential-phase *E. mundtii* and *Enterococcus faecalis* cells secrete ATP more efficiently than do stationary-phase cells. Other bacteria, including *Pseudomonas aeruginosa*, *E. coli*, and *S. aureus*, also secrete ATP in the exponential phase but not in the stationary phase. These results suggest that various gut bacteria, including commensals and pathogens, secrete ATP at any growth phase and modulate immune cell function.

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Reviews and Books

Mizunoe Y. What is biofilm? (in Japa-

Department of Public Health and Environmental Medicine

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

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

General Summary

Our major research projects in the 2012 academic year focused on: 1) analysis of oxidative DNA damage, 2) evaluation of mutagenic potential related to diabetes mellitus, 3) The method for analysis of 8-hydroxy-deoxyguanosine with matrix-assisted laser desorption ionization-time of flight mass spectrometry, 4) Evaluation of fatigue following compressed air work using human herpes virus (HHV) 6 in saliva, 5) evidence-based medicine (EBM), 6) a questionnaire survey on drug information, 7) prevalence of menopausal symptoms, 8) associations between body weight and cardiovascular risk factors, 9) ecological studies of suicide, 10) annual changes in the suicide mortality rate in Japan, 11) effects of L-carnosine and its zinc complex polaprezinc on the healing of pressure ulcers, 12) long-term follow-up study of patients with type 2 diabetes, and 13) mental health in the workplace.

Research Activities

Experimental Medicine

1. Effects of zinc-excess ingestion on blood coagulation in Sprague-Dawley rats
Humans are in a zinc-subdeficient state in Japan. Therefore, zinc supplements are commercially available. To date, there have been few reports of toxicity due zinc excess. In our previous studies, hemorrhagic tendency was observed in rats fed a high-zinc diet. Therefore, we focused on blood coagulation in rats fed a high-zinc diet.
2. Potential mechanisms responsible for tubulointerstitial nephropathy induced by fluoride in rats with unilateral ureteral obstruction
Fluoride, an environmental pollutant, is excreted from the kidney. In our previous animal experiments, ICR-derived glomerulonephritis mice, which have impaired renal function, were more severely affected by fluoride. In this study, we used rats with unilateral ureteral obstruction causing tubulointerstitial fibrosis. We examined whether fluoride exacerbates tubulointerstitial nephropathy in rats with unilateral ureteral obstruction.
3. A study of decompression stress in hyperbaric work
Decompression stress from hyperbaric work has been evaluated with the Doppler bubble detection technique. We attempted to evaluate decompression stress by means of HHV-6 in saliva along with the Doppler technique. The number of HHV-6 DNA in saliva was well correlated with the results of Doppler bubble detection. We were able to use HHV-6 in saliva as a marker to evaluate decompression stress.

Epidemiology, EBM, investigation, and medical informatics

1. Epidemiology, EBM, investigation, and medical informatics

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 with an explosion of epidemiologic activity covering a wide range of health problems. Our greatest concern is to clarify risk factors for diseases in adults and for intractable diseases. We also studied the methods of medical informatics education and EBM.

2. A questionnaire survey on drug information

A self-administered questionnaire was distributed to approximately 2000 health examinees at a Japanese healthcare center to examine the association between health literacy and information-seeking behavior.

3. Menopause Week questionnaire surveys

Questionnaire surveys were conducted among women aged 45 to 59 years who were registered with an Internet survey company. We described ways of coping with menopausal symptoms. Moreover, we revealed the determinants of quality of life in Japanese middle-aged women.

4. Changes in the prevalence of cardiovascular risk factors in Japanese workers

Using annual health examination data from 2001 to 2011, we described the 10-year changes in the prevalence of cardiovascular risk factors in Japanese workers.

5. Ecological studies of suicide

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

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

We performed a nonrandomized controlled trial to determine the effects of L-carnosine and its zinc complex polaprezinc on the healing of pressure ulcers. The results suggested that L-carnosine and polaprezinc accelerate the healing of pressure ulcers to a similar degree over 4 weeks.

7. Mental health in the workplace

Mental health in the workplace is increasingly recognized as a serious problem. Several questionnaires have been used in attempts to prevent mental illness in Japan. The concrete questions in questionnaires are important for managing stress in the workplace. The purpose of this study was to investigate stress in the workplace using a new questionnaire.

8. Relationships of visit-to-visit variability and time-to-effect in systolic blood pressure to the risks of nephropathy and retinopathy in type 2 diabetes

We investigated whether systolic blood pressure variability can predict the progression of nephropathy and retinopathy in patients with type 2 diabetes and also evaluated the time-to-effect relationship between systolic blood pressure control and the risks of progression of nephropathy and retinopathy.

9. Analysis of local factors and changes in the suicide mortality rate in all prefectures

In 1998, the number of suicides in Japan increased sharply and has exceeded 30,000

for 13 consecutive years since. Regional differences in suicide rates have been reported. This study analyzed the relationship between local factors and the suicide mortality rate (in 1990, 1995, 2000, and 2005) classified by prefecture and sex.

Publications

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mycin administration. *Mutat Res.* 2012; **747**: 138-41.

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Department of Forensic Medicine

Kimiharu Iwadate, *Professor*
Kyoko Maebashi, *Assistant Professor*

Kenji Fukui, *Assistant Professor*

General Summary

Our main research projects in 2012 have focused on analysis of the ubiquitin proteasome system and the autophagy lysosome system in the central nervous system, identification of war-dead remains through DNA analysis, the objective evaluation of the limits of DNA typing based on the intensity of ninhydrin treatment, and quantitative analyses of medicines and poisonous substances in forensic autopsy cases.

Research Activities

Forensic pathology

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

Research associated with the ubiquitin proteasome system and the autophagy lysosome system, which play major roles in the degradation of intracellular proteins and organelles, has recently advanced in various areas of medical science. Tissue obtained at autopsies performed at the Department of Forensic Medicine in cases of traumatic intracranial injury were examined to investigate how the ubiquitin proteasome system and autophagy lysosome system are induced in traumatic intracranial injury. We found that the degradation of both pathways was induced in the injured cortex soon after trauma; this finding suggests that the pathway involved in the degradation of unnecessary substances or cells where degradation is activated may differ or change over time after the traumatic event in the central nervous system. Furthermore, beading of the astrocytic processes (clasmato-dendrosis) following head trauma was associated with the protein degradation pathways.

DNA analysis

1. Identification of war-dead remains with DNA analysis

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

2. The objective evaluation of the limits of DNA typing based on the intensity of ninhydrin treatment

Shed epithelial cells on a sheet of paper were stained with ninhydrin reagent, and DNA typing was performed. We studied the relationship between the intensity of the purple staining after ninhydrin treatment and the limits 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. Examination of the DNA extraction method from oral mucosa cells

We examined a simple and easy method for extracting DNA from a living body. Oral mucosa cells were collected from liquid discharged by subjects after they rinsed their mouths. We examined a DNA extraction method to apply commercial DNA extraction kits, and to react oral mucosa cells at constant temperature in one kind of reaction solution for only 10 minutes. A suppressant effect of the DNA extract on an inhibitor of the polymerase chain reaction was confirmed.

Forensic toxicology

1. Quantitative analyses of medicines and poisonous substances

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

2. Examination of a method for analyzing nitrous acid and nitric acid

We detected nitrous acid and nitric acid in an autopsy case. Qualitative and quantitative methods of analyzing nitrous acid and nitric acid with gas chromatography/mass spectrometry were examined. With quantitative analysis, high concentrations of nitrous acid and nitric acid were detected. The results were useful for determining causes of death.

Radiocarbon analysis

1. Establishment of age estimation

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

Publications

Matsumoto S, Iwadate K, Aoyagi M, Ochiai E, Ozawa M, Asakura K. An experimental study on the macroscopic findings of ligature marks using a murine model. *Am J Forensic Med Pathol.* 2013; **34**: 72-4.

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Sakai K, Saito K¹, Takada A¹, Fukunaga T², Iwadate K (Saitama Med Univ, ²Tokyo Med Exam Ofc). Useful pathological features for the

diagnosis of endocardial fibroelastosis (EFE) without congenital cardiac abnormalities: a case of sudden infant death. *Hoigaku no Jissai to Kenkyu.* 2012; **55**: 229-34.

Ochiai E, Fukui K, Maebashi K, Sakai K, Nagai T, Aoyagi M, Iwadate K. Objective assessment of the lower limit of ninhydrin color reaction intensity required for DNA typing of epithelial cells on paper surfaces. *Hoigaku no Jissai to Kenkyu.* 2012; **55**: 235-40.

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Department of Tropical Medicine

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

Kenji Ishiwata, *Associate Professor*

General Summary

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

Research Activities

Alterations of mucins in mice infected with gastrointestinal helminths

Mucus, of which a major constituent is high molecular weight glycoproteins called mucins, is a primary line of defense for the gastrointestinal mucous membrane. Previously we suggested the possibility that interleukin 13-mediated sialylation of mucins is associated with expulsion of adult *Nippostrongylus brasiliensis* in mice. Here we verified the universality of the above association in other gastrointestinal helminths dwelling in the small intestine of mice: *Heligmosomoides polygyrus* (a nematode) and *Vampirolepis nana* (a cestode). The amount of mucins increased 300% in the jejunum, the habitat of *N. brasiliensis*, at the time of worm expulsion, and the mucins reacted with an antibody (HCM31) against sialo sugar chains. Primary infection with *H. polygyrus* results in chronic infection, whereas secondary infection, performed 4 weeks after deworming the primary infection, terminates within 2 weeks. In *H. polygyrus* infection, the amount of mucins increased 300% to 500% in the jejunum, the major habitat of *H. polygyrus*, and the mucins reacted with HCM31 regardless of the number of times of infection. In *V. nana* infection, the amount of mucins increased in the ileum, the habitat of adult *V. nana*, at the time of worm expulsion, and the mucins reacted with an antibody (PGM34) against the sulfo sugar chain. These results suggest that nematode infection induces sialomucins and that cestode infection induces sulfomucins, although these changes do not appear to be consistently involved in worm expulsion. Further study is needed to confirm our findings.

Transcriptome analysis of Entamoeba with an ultrafast sequencer

We have been performing transcriptome analysis of *Entamoeba histolytica* and *Entamoeba invadens*, which are parasitic amoebas of humans and reptiles, respectively, and have similar morphology and life cycle. *E. invadens* can be easily induced to undergo encystation in an *in vitro* axenic liquid culture and has been used as an alternative model

for the encystation of *E. histolytica*. Using oligo-capping methods (full-length complementary DNA sequence and transcription initiation site [TSS] sequences), we have determined the TSSs of the messenger (m) RNA precisely in large quantities, although TSSs were distributed in clusters and not individually determined. The 5' untranslated regions of mRNA of these *Entamoeba* species are comprehensively short (12.38 nt and 8.15 nt on average, in 37% and 25% of total predicted genes, respectively). Here we searched the base preference and the conserved motifs, which are position-specific to TSSs. Among the clusters of TSSs, purines (guanine or adenine) were predominant (purine:pyrimidine ratio = 9:1). More than 70% of nucleotides at the most frequent TSS (at the position 0) in a TSS cluster were adenines, and more than 80% of nucleotides at the position -1 were pyrimidines. We found and characterized 5 motifs: AACT and TAT(A/T)(T/A)AA were position-specific to TSSs; AACCT and AGGGTT were complementary to each other; and many genes with the GGAA motif were specifically expressed during encystation of *E. invadens*.

A simple procedure for permanently stained preparations of E. histolytica and Giardia intestinalis in stool samples using BD SurePath™

BD SurePath™ (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) is new liquid-based cytology system in which negatively charged cells are “smeared” on a positively charged precoated glass slide. By modifying several steps of the BD SurePath™ system, which ordinarily used with Papanicolaou staining, we can easily make trichrome-stained permanent preparations of cysts of *E. histolytica* and *G. intestinalis*. First, stool samples were suspended in the basic solution for Kohn's stain and left to fix for more than 10 minutes. Then the sample was placed in a chamber settled on the precoated (positively charged) glass slide. After the chamber was removed, the slide was immersed in 70% ethanol and stained with trichrome stain. As a result, stool samples were thinly smeared on the slides. Internal structures, such as the nucleus and karyosome, stained clearly. With conventional direct methods, smearing samples thinly and uniformly is difficult; as a result, the morphology of protozoa are difficult to see in the thick part of the smear, and staining is often irregular. In addition, making smears is time-consuming, stools cannot be smeared several days after being passed, and smears of watery stool easily become detached. We were able to overcome these drawbacks by using the BD SurePath™ system.

Molecular interplay between malaria liver stage parasites and the host

Malaria remains a major global health burden, infecting more than 300 million people and resulting in approximately 1 million deaths each year. This grim situation has led to the search for novel control and intervention strategies, in particular, the development of a malaria vaccine. However, these efforts have met with limited success because of the antigenic complexity of the parasite and the differential expression of proteins. Therefore, the next generation of malaria vaccine might exploit a variety of methods for activating an immune response. To better understand the molecular interplay between malaria parasites and hosts for future vaccine development, we have focused on malaria liver stage development, especially the parasitophorous vacuole membrane (PVM), which

is the foremost parasite membrane in the battle against the host. We have found that liver stage parasite PVMs were surrounded by the host cell autophagy marker (LC3) in early liver stage development (< 24 hours after infection); however, most parasites had survived without digestion. Possibly the malaria parasite can control the host cell autophagy response that accompanies digestion. These findings reveal a novel parasite-host interaction and suggest that malaria liver stage parasites possess escape/control/hijack molecules against host digestion for survival inside the host cell.

Differential dynamics of host amino acids in plasma and liver during infection of malaria parasite Plasmodium yoelii

Although malaria is the most significant human parasitic disease, our understanding of the energy metabolism of the principle pathogen, *Plasmodium falciparum*, remains incomplete. Amino acids have long been known to be essential nutrients, and much of the current knowledge of *Plasmodium* energy metabolism is based on early biochemical work, performed with basic analytical techniques, almost exclusively on human plasma with considerable interindividual variability. To further characterize the fate of amino acid metabolism in the malaria parasite, multivariate analysis using statistical modeling of amino-acid concentrations (aminogram) of the plasma and liver were performed in hosts infected with the rodent malaria parasite, *P. yoelii*. Comprehensive and statistical aminogram analysis revealed that *P. yoelii* infection caused marked changes in plasma and liver aminograms and altered the intracorrelation and intercorrelation of amino acid concentrations in the plasma and liver. These findings of the interactions between nutrition and *Plasmodium* infection may provide insight into the protective mechanisms and lead to nutrient-based interventions as low-cost and effective adjuncts to current methods of malaria prevention and treatment.

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

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

Odor-based mechanical transmission of bacteria by fly feces

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

Publications

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Department of Laboratory Medicine

Satoshi Kurihara, *Professor*
Akihiro Ohnishi, *Professor*
Ken Kaito, *Associate Professor*
Kenichi Sugimoto, *Associate Professor*
Midori Kouno, *Assistant Professor*

Masato Suzuki, *Professor*
Hiroshi Yoshida, *Professor*
Hironari Sue, *Associate Professor*
Tomokazu Matsuura, *Associate Professor*
Setsuko Akizuki, *Assistant Professor*

General Summary

Research projects in our department in 2012 were concerned with clinical physiology, clinical microbiology, clinical chemistry, hematology, cardiology, clinical cell biology, and clinical psychiatry. Research achievements in each division are described below.

Research Activities

Clinical physiology

This study examined the effects of long-term caffeine intake and regular exercise, alone and in combination, on triglyceride and glycogen contents in the liver and skeletal muscle, an indicators of insulin resistance. Otsuka Long-Evans Tokushima fatty rats were divided into sedentary, exercise, caffeine-intake, and caffeine-intake and exercise groups for 5 weeks of treatment from 25 to 29 weeks of age. The caffeine-intake group and the caffeine-intake and exercise group were fed rat chow containing 0.25% caffeine, and the exercise group and the caffeine-intake and exercise group were encouraged to exercise every day. Treatment with caffeine only, exercise only, or both caffeine and exercise reduced body weight, visceral fat mass, and metabolic syndrome-related variables. Combination of long-term caffeine intake and exercise expenditure more effectively decreases insulin resistance in both the liver and skeletal muscles and risk factors for metabolic syndrome in Otsuka Long-Evans Tokushima fatty rats than does caffeine intake or exercise alone.

Clinical microbiology

Several clinically isolated, previously unidentified bacterial strains were identified though gene sequencing of polymerase chain reaction-amplified 16S ribosomal RNA. Pantón-Valentine leukocidin (PVL) secreted by *Staphylococcus aureus* causes severe infections of the skin, soft tissue, and lung. To assess the prevalence and genetic characteristics of PVL-positive *S. aureus*, we investigated 86 *S. aureus* isolates obtained from skin and soft-tissue pus from September 2011 through May 2012 at Daisan Hospital. A total of 6 PVL-positive strains (6.9%) were detected: 3 methicillin-sensitive *S. aureus* isolates and 3 methicillin-resistant *S. aureus* isolates. The PVL prevalence was 11.1% in methicillin-resistant *S. aureus* and 5.1% in methicillin-sensitive *S. aureus*.

Clinical chemistry

1. Successful eradication of *Helicobacter pylori* is extremely important for preventing

the progression of gastroduodenal diseases. A triple regimen combining a proton-pump inhibitor (PPI) and 2 antibiotics (amoxicillin and clarithromycin or metronidazole: the first-line treatment) is now considered the gold standard of therapy for eradicating *H. pylori* in Japan. However, the eradication rate of the first-line treatment is only 70% to 85% and has tended to decrease because of increasing rates of resistance to clarithromycin and metronidazole. In the present study we examined the susceptibility of *H. pylori* to antibiotics and a PPI using *in vitro* determination of the minimum inhibitory concentration (MIC) and genetic polymorphism of cytochrome P450 2C19 (CYP2C19), an enzyme that metabolizes PPIs, in 28 patients with *H. pylori* infection and dyspeptic diseases in whom the first-line eradication therapy had failed. The rate of resistance, on the basis of MIC, was 64% (18 of 28 *H. pylori* isolates) to clarithromycin and 52% (14 of 27 isolates) to metronidazole. On the basis of these results we have chosen a new regimen of antibiotics and a PPI for eradicating *H. pylori*. With this new regimen second-line or third-line therapy successfully eradicated *H. pylori* in 20 of 28 patients (72%). According to the CYP2C19 genotype, the eradication rate was 100% (6 of 6 patients) with the CYP2C19 homozygous poor metabolizer genotype, 69.2% (9 of 13 patients) with the heterogenous extensive metabolizer genotype, and 55.6% (5 of 9 patients) with the homozygous extensive metabolizer genotype. Use of MIC susceptibility to select the drug regimen increased the eradication rate, even in patients who had failed first-line eradication therapy. Such an increase in the eradication rate appears to depend on CYP2C19 genetic polymorphism, although the mechanism remains unclear.

2. Our principal research interests are to clarify the pathophysiology of atherosclerosis in relation to impaired lipoprotein metabolism and oxidized low-density lipoprotein (LDL) and to develop methods of assessing cardiovascular disease risk, including the application of our high-performance liquid chromatography (HPLC) method to determine cholesterol levels of lipoproteins.

We investigated and reported the following. 1) The HPLC method we developed can provide excellent quantitative performance for lipoprotein cholesterol in samples with increased remnant lipoprotein (*Atherosclerosis*, 2012 Jun; 222(2): 541-4). 2) Intermediate-density lipoprotein cholesterol may serve as a useful marker for the risk of coronary heart disease risk in Japanese men with increased levels of non-high-density lipoprotein (HDL)-cholesterol (*Int J Cardiol*, in revision). 3) Pleiotropic effects of hydroxymethyl glutaryl coenzyme A reductase inhibitors (statins) on oxidized lipoproteins are divergent, and pitavastatin can markedly decrease malondialdehyde-modified LDL/apolipoprotein B, whereas atorvastatin can decrease oxidized HDL/apolipoprotein A1 (*Atherosclerosis*, in press). (4) An LDL cholesterol homogenous assay agrees with the LDL-C beta-quantification assay in healthy subjects but exhibits positive bias for subjects with hypertriglyceridemia among unhealthy subjects for some reagents (*Atherosclerosis*, 2012 Jun; 225(1): 208-15).

Hematology

We evaluated the efficacy of the body-fluid (BF) mode of an automated hematology analyzer in measuring leukocyte counts in samples of whole blood that contain only a

few leukocytes. Although in some cases leukocyte counts falsely increased in the BF-mode with a Flag message, there was a good correlation between BF mode and eye count. These results indicate that the BF mode of an automated hematology analyzer is useful for counting leukocytes in samples of whole blood with very few leukocytes if we pay attention to the Flag message.

Cardiology

We researched 2 topics in 2012. One topic was the meaning of T-wave abnormalities on electrocardiograms, and the other was the recurrence of atrial fibrillation after pulmonary vein isolation. We published several papers about new methods for preventing the recurrence of atrial fibrillation after pulmonary vein isolation.

Clinical cell biology

1. We developed the fasting ^{13}C -glucose breath test to evaluate the insulin resistance of the liver. In the present study, we attempted metabolic simulation with a computer to estimate *in vivo* glucose metabolism from the dynamics curve of the ^{13}C excretion rate on the fasting ^{13}C -glucose breath test. First, we established an *in vivo* metabolic model of glucose and adopted 5 compartment models. The dynamics curve of the exhalation ^{13}C excretion by 5 compartment models fitted well that with the actual value. In healthy subjects, the area under the curve until 360 minutes of the ^{13}C excretion dynamics curve of female subjects was greater than that of male subjects. The *in vivo* simulation suggested that the glucose metabolism in the liver of female subjects is more efficient than that in male subjects. This simulation is also useful for judging the effects of drugs before and after treatment. (Supported by a Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Strategic Research Foundation at Private Universities, 2011-2015) (In collaboration with Meiji University, the National Defense Medical College, and the Departments of Internal Medicine and Surgery, The Jikei University)
2. We have developed a novel, quantitative, and specific assay of plasma latency-associated protein of transforming growth factor β degradates (LAP-D), which are produced during proteolytic activation of transforming growth factor β . The level of LAP-D in blood and tissues would be a novel marker reflecting fibrogenesis activity but not the amount of accumulated fibrosis in patients. We found that even if nucleotide analogs inhibited alanine aminotransferase release from livers infected with hepatitis B virus, the plasma LAP-D level increased in some cases. Most likely, the effect of nucleotide analogs is not sufficient to inhibit fibrogenesis in the liver. (Supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation and the Research on the Innovative Development and the Practical Application of New Drugs for Hepatitis B provided by the Ministry of Health, Labour and Welfare of Japan) (In collaboration with the Institute of Physical and Chemical Research)

Clinical psychiatry

We reported a case of epilepsy in a patient with ring chromosome 20 syndrome, and

from a review of the literature, we discussed the characteristics of patients with ring chromosome 20 syndrome. Furthermore, we reported a case of elderly-onset partial epilepsy with manic state due to postictal psychosis. A study was performed to prevent the recurrence of depression in patients with epilepsy. We are planning a study of the management of epilepsy in pregnant women.

Publications

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Department of Internal Medicine

Division of Gastroenterology and Hepatology

Hisao Tajiri, *Professor*
 Mikio Zeniya, *Professor*
 Ichiro Takagi, *Professor*
 Hirokazu Nishino, *Professor*
 Tomohisa Ishikawa, *Associate Professor*
 Mika Matsuoka, *Assistant Professor*
 Satoshi Suto, *Assistant Professor*
 Seiji Arihiro, *Assistant Professor*
 Hiroshi Abe, *Assistant Professor*
 Masayuki Saruta, *Assistant Professor*

Mariko Itsubo, *Professor*
 Toshifumi Okusa, *Professor*
 Yoshio Aizawa, *Professor*
 Shigeo Koido, *Associate Professor*
 Atsushi Hokari, *Associate Professor*
 Kazuhiko Koike, *Assistant Professor*
 Yoshinari Miyagawa, *Assistant Professor*
 Yasushi Onoda, *Assistant Professor*
 Shinichiro Uetake, *Assistant Professor*

Research Activities

Alimentary Tract

1. An antibody against tumor necrosis factor α is effective at preventing restenosis after endoscopic balloon dilatation therapy in patients with Crohn's disease

We enrolled 12 patients with Crohn's disease who had severe stenotic lesions. Seven patients had already been treated with infliximab or adalimumab before endoscopic balloon dilation (EBD), and 5 patients had started being treated with infliximab or adalimumab after EBD. No patients had symptoms of ileus, but 4 patients underwent EBD again because of mild restenosis. Endoscopic examination showed no restenosis in 4 patients. Our findings show that EBD is a useful treatment for stenosis in Crohn's disease and that an antibody against tumor necrosis factor α is useful for preventing restenosis after EBD.

The cell membrane fatty acid profile of patients with Crohn's disease at initial onset was significantly different from those of healthy subjects and patients with initial-onset ulcerative colitis (UC). This finding suggests that the lipid metabolism enzyme, i.e., delta 6-desaturase, is hyperactivated in Crohn's disease.

2. Development of optical molecular imaging for gastrointestinal cancers and image-guided phototherapy

We have developed photoimmunotherapy, a type of specific molecularly targeted phototherapy that uses monoclonal antibodies conjugated with near-infrared phthalocyanine dye. We have recently established a technique of specific molecularly targeted, fluorescence molecular imaging-guided phototherapy using a mouse model of human gastric cancer.

3. Endoscopic features of colorectal serrated lesions using image-enhanced endoscopy with pathological analysis

Sessile serrated adenoma/polyp (SSA/P) has recently been reported to have malignant potential. Color change on autofluorescence imaging, capillary dilatation, presence of a mucous layer on the tumor surface, and pit dilatation under narrow-band imaging were examined retrospectively. When magenta color, capillary dilatation, mucous cap, and pit dilatation were used for differentiating SSA/P from hyperplastic polyp, the sensitivity,

specificity, and accuracy were 43%, 68%, and 52%, respectively, for autofluorescence imaging; 10%, 96%, and, 41% for capillary dilatation; 94%, 40%, and 75% for mucous cap; and 80%, 72%, and 78% for pit dilatation. The findings of a mucous cap and a dilated pit might be helpful for differentiating SSA/P from hyperplastic polyp.

4. Elemental diet as preparation for colonoscopy in inflammatory bowel disease

We investigated the usefulness of an elemental diet as a bowel preparation regimen for colonoscopy in inflammatory bowel disease (IBD). One day before colonoscopy, patients with IBD received 1,200 ml of an elemental diet. A polyethylene glycol (PEG) lavage solution was used to cleanse the colon. The total amount of PEG solution used, the degree of colonic cleansing achieved, patient acceptance, and the safety of the regimen were evaluated. Bowel preparation with an elemental diet significantly reduced PEG volume for gut lavage before colonoscopy and was effective, safe, and suitable for patients with IBD.

5. Photodynamic diagnosis of colitis-associated dysplasia in a mouse model after oral administration of 5-aminolevulinic acid

Early and accurate detection of dysplastic lesions has become an important issue in the treatment of patients with chronic, extensive UC. We detected dysplastic lesions in mouse colonic tissue by determining the accumulation of the photosensitizer protoporphyrin IX induced by the oral administration of 5-aminolevulinic acid, a precursor of protoporphyrin IX. Photodynamic diagnosis with 5-aminolevulinic acid will be useful in detecting dysplastic lesions in the colon mucosa of patients with UC.

6. Efficacy of enemas with a zinc-containing compound, polaprezinc, in patients with UC

Polaprezinc enemas produced significant improvement, as assessed with endoscopic and clinical findings, in cases of moderate-to-severe UC. Significant improvements were detected with endoscopy in the rectum and sigmoid colon, which are areas reached by polaprezinc enemas. Polaprezinc enemas are a useful new add-on treatment to accelerate mucosal healing in UC.

7. Side effects of azathioprine

A pyrophosphohydrolase (thiopurine S-methyltransferase) gene mutation, 94C>A, was detected in most cases of leukopenia. However, this mutation might not definitively reflect the risk of adverse reactions to thiopurines.

8. Novel immunotherapy for pancreatic cancer

Fusions of dual Toll-like receptor-stimulated dendritic cells and ethanol-treated tumor cells induced efficient cytotoxic T lymphocytes through transforming growth factor β 1 blockade and interleukin 12p70 production.

Liver

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

Resistant viral mutations are an urgent remedial problem in chronic hepatitis B virus (HBV) infection treated with antiviral analogue nucleic acids. Analyses were performed of the viral gene sequence and the treatment response. We have considered the possibility of a new concurrent therapy for chronic HBV infection.

2. The relation histological findings and biochemical laboratory data of chronic hepatitis C virus infection

Histological activity and staging in some cases of chronic hepatitis C virus (HCV) infection did not match the biochemical data. We must weigh histological findings against biochemical data.

3. Clinical pathological study of primary biliary cirrhosis

We studied the clinical characteristics of primary biliary cirrhosis. We attempted to statistically analyze blood chemical variables and histological findings of liver biopsy. A comparison of clinical characteristics and micro-RNA expression in T cells was started this year.

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

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

5. Nutritional imbalance in patients with liver cirrhosis

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

6. Nutritional evaluation in nonalcoholic fatty liver disease

The pathogenesis of nonalcoholic fatty liver disease is similar to that of metabolic syndrome. We evaluated nutritional conditions in detail in nonalcoholic fatty liver disease and metabolic syndrome. We have explored the possibility of a new nutrition supports system with accuracy.

7. A study comparing sleep apnea and nonalcoholic fatty liver disease

Sleep apnea syndrome seems to be closely related with an imbalance of hepatic metabolism. Sleep apnea syndrome is often complicated by severe hypoxia and liver dysfunction. We analyzed the respiratory quotient with indirect calorimetry.

8. Prognostic indices in hepatocellular carcinoma

The serum white blood cell count and the serum albumin concentration before treatment were independently associated with overall survival. We found that the values of these variables before treatment were associated with tumor progression and reduced liver function and were independent markers of poor prognosis in patients with hepatocellular carcinoma.

9. The features of dyslipoproteinemia in chronic HCV infection

The efficacy of PEG-interferon plus ribavirin combination therapy or telaprevir and PEG-interferon plus ribavirin triple therapy on chronic HCV infection was examined. In addition, the significance of interleukin (IL) 28B (rs8099917) single nucleotide polymorphism, HCV mutations, and dyslipoproteineima on the efficacy of antiviral therapy was determined. Of these factors, IL28B single nucleotide polymorphism was extremely important for predicting therapeutic outcome in patients with HCV genotype 1b. With triple therapy, almost all patients with genotype 1b carrying the IL28B major genotype achieved a sustained virological response. Meanwhile, almost all patients infected with HCV genotype 2 could achieve a sustained virological response with

response-guided therapy.

10. The fasting ^{13}C -glucose breath test

We attempted metabolic simulation with a computer to estimate *in vivo* glucose metabolism from the dynamics curve of the ^{13}C excretion rate on the fasting ^{13}C -glucose breath test. First, we established an *in vivo* metabolic model of glucose and adopted 5 compartment models. The dynamics curve of the exhalation of ^{13}C excretion in the 5 compartment models fitted well with that of actual values. In healthy subjects, the area under the curve until 360 minutes of the ^{13}C excretion dynamics curve in female subjects was greater than that in male subjects. The *in vivo* simulation suggested that glucose metabolism in the liver of female subjects is more efficient than that in male subjects. This simulation is also useful for judging the effects of drugs before and after treatment.

11. A novel, quantitative, and specific assay of plasma latency-associated protein of transforming growth factor degradates

The level of latency-associated protein of transforming growth factor β degradates (LAP-D) in blood and tissues would be novel markers reflecting fibrogenesis activity but not the amount of accumulated fibrosis in patients. We found that even if nucleotide analogs inhibited alanine aminotransferase release from livers infected with HBV, the plasma LAP-D level increased in some cases. Most likely, the effect of nucleotide analogs is not sufficient to inhibit fibrogenesis in the liver.

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Department of Internal Medicine

Division of Neurology

Yasuyuki Iguchi, *Professor*
 Akira Kurita, *Associate Professor*
 Masahiko Suzuki, *Assistant Professor*
 Chizuko Toyoda, *Assistant Professor*
 Renpei Sengoku, *Assistant Professor*

Hisayoshi Oka, *Professor*
 Kazutaka Matsui, *Assistant Professor*
 Hiroshi Yaguchi, *Assistant Professor*
 Yu Kono, *Assistant Professor*

General Summary

Our clinical research in 2012 was conducted in the following areas: 1) neurodegenerative disease, 2) cerebrovascular disease, 3) peripheral neuropathy, 4) myasthenia gravis, and 5) migraine. We also performed basic research in the following areas: 1) neuroanatomy, 2) motoneuron disease, and 3) lipid metabolism in cerebrovascular disease.

Research Activities

Neurodegenerative disease

1. Evaluation of gastrointestinal function according to the Gastrointestinal Symptom Rating Scale in Parkinson's disease and the effect of nizatidine on gastrointestinal symptoms

We studied the relations of the Gastrointestinal Symptom Rating Scale scores to clinical characteristics and indices of autonomic dysfunction, such as cardiac ¹²³I-metaiodobenzylguanidine uptake, heart rate variability, and orthostatic hypotension, in 28 patients with Parkinson's disease (PD). We found that patients with PD who responded to nizatidine tended to have milder cardiovascular autonomic dysfunction than did nonresponders.

2. 25-Hydroxyvitamin D, vitamin D receptor gene polymorphisms, and the severity of PD

We examined associations among serum levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, vitamin D receptor polymorphisms, vitamin D-binding protein gene polymorphisms, and the severity of PD. We found that higher levels of 25-hydroxyvitamin D and the vitamin D receptor FokICC genotype were independently associated with milder forms of PD.

3. The Odor Stick Identification Test for the Japanese can be used to differentiate PD from multiple system atrophy and progressive supranuclear palsy

We assessed olfactory function with the Odor Stick Identification Test for the Japanese (OSIT-J) in 94 Japanese patients with idiopathic PD, 15 with multiple system atrophy with predominant parkinsonism, 7 with progressive supranuclear palsy, and 29 age-matched control subjects. The OSIT-J is a potentially useful clinical test for detecting olfactory deficits in PD and for differentiating PD from progressive supranuclear palsy and multiple system atrophy with predominant parkinsonism.

4. Differences in fatigue and clinical features of the subtypes of PD

We compared the differences among fatigue and various clinical features of PD subtypes, using the 16-item Parkinson Fatigue Scale. We found that the akinetic rigid type of PD differs from the tremor-dominant type in motor impairment, fatigability, and autonomic failure.

5. Measurement of olfactory bulb volume is useful for the differential diagnosis of neurodegenerative diseases

We investigated the volume of the olfactory bulb in PD and PD-related diseases. We found that the olfactory bulb volume in patients with PD was smaller than that in patients with PD-related diseases.

6. Dysphagia and vocal cord palsy in multiple system atrophy

We assessed dysphagia and vocal cord palsy laryngoscopically in multiple system atrophy. Many patients had vocal cord palsy at the time of percutaneous endoscopic gastrostomy. We suggest that vocal cord palsy should be assessed before percutaneous endoscopic gastrostomy is performed.

7. Direct comparison of *in vivo* accumulation of 2 amyloid imaging probes, [¹¹C] Pittsburgh compound B and [¹¹C] BF227, in Alzheimer's disease

The purpose of this study was a direct comparison of the characteristics of 2 amyloid probes, [¹¹C] Pittsburgh compound B and [¹¹C]BF227, in the same patients with Alzheimer's disease. We found that the difference in the distribution of the 2 probes presumably reflects the difference in the specificity to amyloid beta or the difference in the affinity to the different stage of amyloid-beta aggregation in the senile plaque generation process or both.

8. Epidemiological study of the progression and prognosis of amyotrophic lateral sclerosis in the northern Tokatsu area of Chiba

Two medical students of The Jikei University performed epidemiological research on the progression and prognosis of ALS. They found that patients in whom lower-extremity weakness had initially developed had the slowest progression among patients with ALS, whereas patients in whom dysphagia had initially developed had rapid progression resulting in respiratory failure.

Cerebrovascular disease

1. Sonothrombolysis in acute ischemic stroke

Our objective was to develop a handy transducer that can safely enhance the thrombolytic activity of recombinant tissue plasminogen activator for acute ischemic stroke. We will be creating an experimental model with bone chips to confirm the penetration of ultrasound through bone.

2. Clinical factors associated with favorable outcomes in acute ischemic stroke treated with intravenous recombinant tissue plasminogen activator

We started the Jikei Stroke Registration to investigate factors associated with favorable and poor outcomes in stroke and to compare the prognosis between stroke with in-hospital onset and stroke with out-of-hospital onset.

3. Stroke incidence in Fabry disease

We will develop a prospective registry of Fabry disease and analyze the factors associated with stroke occurrence. We will also calculate the crude incidence rate of first-ever

stroke in patients with Fabry disease.

4. Evaluation of the severity of periventricular hyperintensity using transcranial color flow imaging

We investigated the relationship between white matter lesions on magnetic resonance imaging and flow variables in the middle cerebral artery, as measured with transcranial color flow imaging. We found that the evaluation of middle cerebral artery flow variables, with the combination of the pulsatility index and end-diastolic velocity, may be useful for predicting periventricular hyperintensity.

5. Clinical characteristics associated with abnormal diffusion-weighted images in patients with transient cerebral ischemic attack

We investigated the clinical characteristics associated with diffusion-weighted image (DWI) positivity in patients with transient ischemic attack. We found that the DWI positivity rate was 35% and that the blood urea nitrogen/creatinine ratio, blood glucose level, and brain natriuretic protein level were significantly higher in DWI-positive patients than in DWI-negative patients.

6. Can eicosapentaenoic acid reduce the progression of arteriosclerosis?

We prospectively investigated whether eicosapentaenoic acid can control the progression of arteriosclerosis. At present, patients receiving eicosapentaenoic acid and a statin show a tendency for slower progression of arteriosclerosis.

Peripheral neuropathy

1. Neurophysiological studies of subclinical diabetic polyneuropathy

The clinical utility of nerve conduction studies and neurological examination of the feet with newly established techniques was assessed in patients with diabetes mellitus, who had neither sensory symptoms in the feet nor autonomic nervous symptoms. We found that even in patients without symptoms of neuropathy, our methods could detect subclinical peripheral or autonomic neuropathy in a certain percent of patients.

Myasthenia gravis

1. Administration period of tacrolimus for patients with myasthenia gravis treated with thymectomy

We have been investigating the administration period of tacrolimus for patients with myasthenia gravis treated with thymectomy.

Migraine

1. Beneficial effects of valproate on the floating feeling and dizziness in patients with long-standing migraine

The effects of valproate on the floating feeling and dizziness were retrospectively investigated in patients with long-standing migraine. We found that valproate was useful for treating these symptoms associated with migraine.

Clinical researches

1. Plasticity of glycinergic synaptic inputs in the hypoglossal nucleus

We investigated the function and development of inhibitory inputs on motoneurons to

better understand selective vulnerability. We found that glycine input increases with age and that glycine receptors ($\alpha 3$) induce presynaptic plasticity.

2. Study of corticospinal tract functions other than voluntary movement

We are investigating the functions of the corticospinal tract, especially sensory function, by using electrophysiological examinations. We are studying the sensory function of the corticospinal tract by using motor-evoked potentials elicited by magnetic stimulation, sensory-evoked potentials, and nerve conduction studies.

3. Establishment of a high-density lipoprotein functional assay

Either bezafibrate or ethyl icosapentate was administered to patients with dyslipidemia. Cholesterol efflux was assessed using each patient's apolipoprotein B-depleted serum. We found that cholesterol efflux was significantly greater in patients receiving bezafibrate than in patients receiving icosapentate.

4. Gene assay of lipid metabolism

The ATP-binding cassette transporter A1 plays a pivotal role in reverse cholesterol transport, and its degradation is inhibited by PDZRhoGEF-activate RhoA. We confirmed that PDZRhoGEF-activate RhoA knock-down with micro RNA decreases apolipoprotein A1 efflux and *in vivo* reverse cholesterol transport.

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Department of Internal Medicine

Division of Kidney and Hypertension

Tatsuo Hosoya, *Professor and Chairperson*
 Tetsuya Kawamura, *Associate Professor*
 Keitaro Yokoyama, *Associate Professor*
 Yoichi Miyazaki, *Assistant Professor*
 Masato Ikeda, *Assistant Professor*
 Takashi Yokoo, *Assistant Professor*
 Ichiro Ohkido, *Assistant Professor*

Iwao Ohno, *Professor*
 Yasunori Utsunomiya, *Associate Professor*
 Makoto Ogura, *Associate Professor*
 Kazushige Hanaoka, *Assistant Professor*
 Hiroshi Hayakawa, *Assistant Professor*
 Nobuo Tsuboi, *Assistant Professor*

General Summary

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

Research Activities

Clinical studies of immunoglobulin nephropathy

We retrospectively analyzed the prognosis of immunoglobulin nephropathy, led a research group of the Ministry of Health, Labour and Welfare, and published a new clinicopathologic classification for predicting long-term renal outcomes. A manuscript describing a randomized clinical trial of steroid pulse therapy with or without tonsillectomy is under peer review. Furthermore, we studied the prognostic factors for steroid-treated immunoglobulin nephropathy and concluded that the achievement of proteinuria <0.4 g/day at 1 year could be a therapeutic indicator for a favorable renal outcome.

Clinical studies of low glomerular density in various glomerular diseases

Our previous studies showed that low glomerular density is strongly associated with the prognosis of various glomerular diseases. Moreover, we reported that low glomerular density with glomerulomegaly was the most characteristic finding in obesity-related glomerulopathy. To collect supporting evidence, we are investigating the distribution of glomerular density in different cortical zones of the human kidney. Furthermore, collaborative research about estimating the number of nephrons in Japanese subjects is in progress.

Experimental study on glomerular epithelial cells

Transgenic mice (NEP25) express human CD25 selectively on podocytes, and injection of a human CD25-targeted recombinant immunotoxin (LMB2) permits selective injury of glomerular epithelial cells (GECs). With this model we are investigating

the mechanisms of GEC regeneration after glomerular injury. Furthermore, we are investigating the role of oxidative stress in GEC injury, with a focus on the Keap1-Nrf2 system, a master regulator of the antioxidant response.

Studies of patients with CKD

In clinical research we found that higher levels of 1,25-dihydroxyvitamin D may be associated with better kidney function in patients with type 2 diabetes and that this association was modified by vitamin D receptor gene polymorphisms. We investigated the effect of cinacalcet on serum levels of calcium and phosphorus in patients undergoing hemodialysis with or without a high parathyroid hormone level to control serum levels of calcium and phosphorus. We concluded that administration of cinacalcet to patients with or without high parathyroid hormone levels facilitates the control of calcium and phosphorus levels. Moreover, we found that serum levels of the sphingolipid sphingosine 1-phosphate decreased significantly in all patients after 6 months of treatment with cinacalcet. In basic research, we analyzed the relation between bone and vascular injury in CKD and evaluated the enzyme klotho. In addition, we found that the serum creatinine level was lower in patients undergoing hemodialysis than in healthy persons. The serum phosphorus level was independently associated with the serum soluble α -klotho level.

Studies of patients undergoing peritoneal dialysis

Encapsulating peritoneal sclerosis is a severe complication of long-term peritoneal dialysis (PD) and has a high mortality rate. We used a laparoscopic approach to evaluate peritoneal injury in patients undergoing PD. We found that PD peritonitis is a risk factor for encapsulating peritoneal sclerosis, and we hypothesized that the bacterial species causing PD peritonitis would change depending on the neutral-pH PD solution.

Studies of renal transplantation

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

Studies of patients with polycystic kidney disease

We investigated the mechanism by which the intracellular Ca^{2+} concentration changes with drug administration or with changes in the extracellular Ca^{2+} concentration. Calcium oscillations may be associated with the function of renal tubular epithelial cells.

Clinical study on hypertensive patients with CKD

The Jikei Optimal Antihypertensive Treatment (JOINT) study is a large-scale prospective interventional observational study investigating the effect of a fixed-dose combination of losartan and hydrochlorothiazide in patients with CKD. The study, which had a total of 280 subjects, has been completed. The main results were published in *Clinical and Experimental Nephrology*. Additionally, we performed an extensional observation on the relationship between uric acid and its associates as a subanalysis of the JOINT study. The results were submitted to the English version of *Internal Medicine*.

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

We examined the relationship between central blood pressure (CBP) and the activity of the renin-angiotensin-aldosterone system (RAS) in patients with primary aldosteronism and in patients with essential hypertension. The gap between CBP and brachial systolic blood pressure (SBP) increased with the plasma aldosterone concentration in patients with essential hypertension. Although patients with primary aldosteronism did not demonstrate this tendency, the CBP-SBP gap in these patients was significantly higher than that in patients with essential hypertension, and this gap decreased with adrenalectomy or eplerenone treatment in patients with primary aldosteronism. This study suggests that, in primary aldosteronism, the regulation of the RAS is impaired by oversecretion of aldosterone and by the increase in CBP by a mechanism independent of the serum RAS, such as elevated organ RAS and inflammation. Furthermore, even if the SBP is controlled normally, the kinetics of the CBP indicate a different tendency from SBP as the RAS increases and might increase the risk of cardiovascular events.

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Department of Internal Medicine

Division of Rheumatology

Daitaro Kurosaka, Associate Professor
Ken Yoshida, Assistant Professor

Isamu Kingetsu, Assistant Professor

General Summary

An internist must aim to practice patient-oriented medicine that is well grounded in medical science. Therefore, our department encourages its staff 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 synovial neovascularization in experimental murine arthritis

Several studies have shown the arthritis-inhibiting effects of neovascularization inhibitors in animal models of rheumatoid arthritis (RA). We examined the effects of the neovascularization inhibitor sunitinib in a murine model of collagen-induced arthritis. Furthermore, we demonstrated in bone marrow-chimeric mice that some synovial endothelial cells, immature pericytes, synovial macrophages, and osteoclasts are derived from bone marrow.

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

To assess synovial neovascularization in patients with RA, we have evaluated the synovial blood flow signals of patients' joints with power Doppler ultrasonography and analyzed their correlation with neovascularization-related factors in serum and other variables of disease activity. We demonstrated that the synovial blood flow level and the serum level of interleukin 6, an inflammatory cytokine, correlate with the serum levels of key neovascularization-related factors vascular endothelial growth factor and angiopoietin 2.

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

Telomerase activation has 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. This year we measured telomerase activity in peripheral blood mononuclear cells obtained from patients with autoimmune diseases, especially RA.

4. Citrullination of chemokines in RA

Citrullination catalyzed by peptidylarginine deiminase is a posttranslational modification of arginine to citrulline, which contributes to the pathogenesis of RA. Assistant Professor Yoshida performed a study at the University of Michigan to examine the presence and functions of citrullinated chemokines in RA. A newly developed

enzyme-linked immunoassay system demonstrated that concentrations of citrullinated epithelial-derived neutrophil-activating peptide 78 (ENA-78)/chemokine (C-X-C motif) ligand 5 (CXCL5) were higher in RA than in other rheumatic diseases, such as osteoarthritis, and correlated with the C-reactive protein level and the erythrocyte sedimentation rate. Although ENA-78/CXCL5 is a neutrophil chemotactic factor, an *in-vitro* chemotaxis assay and an *in-vivo* experiment showed that citrullinated ENA-78/CXCL5 has a monocyte-recruiting function and stimulates inflammation in a model of inflammatory arthritis. Furthermore, citrullinated ENA-78/CXCL5 recruited monocytes via chemokine (C-X-C motif) receptor 2 (CXCR2), which is the primary receptor for ENA-78/CXCL5, and CXCR1, which has 78% homology at the amino-acid level with CXCR2.

Department of Internal Medicine

Division of Cardiology

Michihiro Yoshimura, *Professor*
Ikuo Taniguchi, *Professor*
Teiichi Yamane, *Associate Professor*
Makoto Kawai, *Associate Professor*
Kimiaki Komukai, *Assistant Professor*
Taro Date, *Assistant Professor*

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

General Summary and Research Activities

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

Clinical research

In clinical research, we have been participating in multicenter collaborative studies, including large-scale clinical studies, and conducting research during routine clinical practice. The Nationwide Gender-based Atherosclerosis Determinants Estimation and Ischemic Cardiovascular Disease Prospective Cohort (NADESICO) Study is a multi-center cooperative prospective cohort study of sex differences in risk factors for arteriosclerotic diseases and prevention, which used computed tomographic examinations of coronary arteries. We have converted patient data, including risk factors and lesion morphology, from catheterization examinations and treatment in various clinical research divisions, into a database and performed a study comparing risk factors, outcomes, and other variables of ischemic heart disease, cardiomyopathy, and other conditions. In addition, we have participated in nationwide clinical studies (J-CRAFT, J-DESERT, CSA, J-LESSON, OPERA trial, NIPPON study, PROPEL study, and REAL-CAD trial), mainly investigating in detail treatment with drug-eluting stents or catheter ablation, and the diagnosis of coronary vasospasm, which is closely related to the etiology of ischemic heart disease.

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

We have been aggressively treating atrial fibrillation by catheter ablation, and during this fiscal year we have treated approximately 220 patients. In addition, in clinical research we have investigated 1) the usefulness of the pulmonary vein antrum isolation procedure by new mapping systems, and 2) optimal catheter ablation strategies for persistent atrial fibrillation.

Basic research

Research activity, such as studies at other institutions in Japan and abroad by graduate students in basic sciences and clinical sciences and presentation of the results of many studies, is being performed.

In the field of arrhythmias, we have performed research in the form of a study of the basis of the development of atrial fibrillation by using various experimental models in regard to the effects of inflammatory cell invasion and fibrosis on the myocardium.

In the field of cardiomyocyte physiology, we have investigated the physiological and pathophysiological regulatory mechanisms of myocardial contraction and relaxation and performed a study with molecular biology techniques and physiological techniques. We have also investigated a new signal-transmission system in the α -receptor stimulation effect in relation to L-type calcium channels in the rat myocardium, the effect of β -receptor stimulation in sarcoplasmic reticulum function, and cardiomyocyte intracellular calcium kinetics in mice in which dilated cardiomyopathy develops because of tropo-nin 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

Kazunori Utsunomiya, *Professor*
Katsuyoshi Tojo, *Professor*
Yutaka Mori, *Associate Professor*
Tamotsu Yokota, *Associate Professor*
Shuichi Kato, *Assistant Professor*
Masaya Sakamoto, *Assistant Professor*

Takashi Sasaki, *Professor*
Kuninobu Yokota, *Professor*
Masami Nemoto, *Associate Professor*
Rimei Nishimura, *Associate Professor*
Kei Fujimoto, *Assistant Professor*
Daiji Kawanami, *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

Several clinical trials of the treatment of type 2 diabetes using continuous glucose monitoring (CGM) are under way. The relationship between glucose fluctuation and diabetic complications is also studied with the data from CGM.

A nationwide epidemiologic study of mortality in approximately 3,500 patients with type 1 diabetes was started in 1986 and has continued to provide much information about the prognosis of Japanese children with type 1 diabetes. A population-based study of childhood obesity and insulin resistance as well as diabetes in elderly and genetic factors has also continued in Niigata Prefecture.

Molecular diabetology

Objective: Spontaneous hypoglycemia occurs owing to several causes with different patterns of hypoglycemia and hormone responsiveness. The aim of this study was to identify gene mutations in a family with spontaneous hypoglycemia by focusing on candidate genes and evaluating metabolism and hormone status.

Methods: The metabolic state was observed with CGM during the starvation test in the proband. Genomic DNA from peripheral blood was sequenced directly to identify gene mutations.

Results: The proband was a 34-year-old woman who was admitted to our university hospital because of severe hypoglycemia and metabolic acidosis associated with diarrhea and loss of appetite. She had had hypoglycemia-like episodes, especially when fasting, since the age of 1 year. In the starvation test, CGM clearly demonstrated no hypoglycemia until 29 hours. However, once hypoglycemia occurred at 29 hours, it persisted even after the induction of glucagon and the suppression of insulin secretion. These findings

strongly suggest that a glyconeogenic enzyme is inactive. Therefore, we focused on key glyconeogenic enzymes, including fructose-1,6-bisphatase (FBP1), phosphoenolpyruvate carboxykinase 1, and pyruvate kinase. The sequencing of these enzymes revealed that the proband and her brother, who had similar hypoglycemia-like episodes, share the same mutant genotype of compound heterozygosity for *FBP1* (G164S/F194S), in which homozygotes of each allele had been reported as a responsible mutation for the phenotype.

Conclusion: Observation of hypoglycemia with CGM and hormone responsiveness in a patient with hypoglycemia permitted a focus on candidate genes and enabled identification of *FBP1* mutations.

Insulin resistance and obesity

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

Dietary therapy

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

Diabetic Vascular Complications

Diabetic complications are major sources of morbidity and mortality in patients with diabetes and an economic burden on societies worldwide. A greater understanding of the molecular targets that regulate both microangiopathy and macroangiopathy could lead to novel therapeutic strategies against diabetic complications. The Rho GTPases and their downstream effectors, Rho-associated kinases (ROCKs), have been implicated as regulators of the actin cytoskeleton. Because changes in the actin cytoskeleton are associated with vascular function, recent studies have revealed that ROCKs play a pivotal role in cardiovascular diseases, such as atherosclerosis, and in vascular remodeling. Accumulating evidence from animal models of diabetes shows that ROCK activity is increased in the kidney, retina, and vessels. Studies using pharmacological inhibition and genetic deletion of ROCKs have demonstrated that ROCK inhibition suppresses diabetic nephropathy by attenuating the excessive production of extracellular matrix induced by diabetes and slows the development of glomerular sclerosis and interstitial fibrosis. Given this background, we investigated the mechanism by which Rho-kinase promotes diabetic nephropathy. It is known that the diabetic kidney is exposed to hypoxic conditions. We found that ROCKs regulate the expression and function of hypoxia-inducible factor 1 α , thereby inducing glomerulosclerosis under diabetic conditions.

Furthermore, we reported for the first time that Rho-kinase regulates endoplasmic reticulum stress-mediated endothelial function.

Finally, we found that fasudil (a Rho-kinase inhibitor) attenuates the progression of diabetic neuropathy in rats with streptozotocin-induced diabetes.

Endocrinology

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

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

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

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

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

Publications

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Reviews and Books

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Department of Internal Medicine

Division of Clinical Oncology/Hematology

Keisuke Aiba, *Professor*
Fumi Mizorogi, *Professor*
Takaki Shimada, *Assistant Professor*
Hidekazu Masuoka, *Assistant Professor*
Kaichi Nishiwaki, *Assistant Professor*
Yuhichi Yahagi, *Assistant Professor*
Yoji Ogasawara, *Assistant Professor*

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

General Summary

The immediate goals of our clinical and basic research are to investigate basic and clinical aspects of malignant diseases and to try to improve outcomes for patients with solid tumors and hematological malignancies, leading to the ultimate goals of improving the natural history of malignant diseases. We have also been performing several clinical trials and basic research studies throughout 2012.

Research Activities

Leukemias

Many patients with previously untreated hematological disorders have been referred to our department. The disorders in 2012 included acute myeloid leukemia (AML), 8 cases; acute lymphoblastic leukemia (ALL), 11 cases; chronic myeloid leukemia (CML), 2 cases; and myelodysplastic syndrome (MDS), 6 cases. We have performed clinical trials as a member of the Japan Adult Leukemia Study Group (JALSG), which is a distinguished leukemia research group established more than 20 years ago in Japan for AML, ALL, and CML. The JALSG protocol studies performed in 2012 were as follows: AML/MDS-HR CS-7 study of newly diagnosed AML; refractory anemia with excess blasts II, all-case registration cohort study; CML-207 (phase III); AML-209-GS; AML209-KIT; and ALL-CS-12. We also participated in several cooperative group studies and pilot studies: Aged Double-7 (newly diagnosed AML in the elderly: phase II), VEGA (MDS: phase II), a study of nilotinib (refractory CML: phase I/II), and a study of dasatinib (refractory CML: phase I/II).

Lymphomas

In 2012 we registered 73 patients with newly diagnosed non-Hodgkin's lymphoma and 2 patient with Hodgkin's lymphoma. We have performed clinical trials as a member of the Lymphoma Study Group of the Japan Clinical Oncology Group (JCOG). The registration of study JCOG0406 (newly diagnosed mantle cell lymphoma: phase II) was completed, and the study JCOG0601 (newly diagnosed low-risk advanced diffuse large B-cell lymphoma: phase II/III) is a still-active protocol study that began in 2007. A randomized phase II study in patients with high-risk diffuse large B-cell lymphoma is also

an on-going protocol study that began in 2010 (biweekly rituximab-cyclophosphamide, doxorubicin, vincristine, and prednisone [R-CHOP] \pm cyclophosphamide, cytarabine, dexamethasone, etoposide, and rituximab [CHASER] vs melphalan, cyclophosphamide, etoposide, and dexamethasone [LEED]; JCOG0908). Other cooperative studies examined biweekly rituximab, etoposide, prednisone, vincristine, hydroxydaunorubicin (R-EPOCH: relapsed and refractory B-cell lymphoma: phase II) and pirarubicin, cyclophosphamide, vincristine, and prednisolone (THP-COP: newly diagnosed T-cell lymphoma: phase II).

Myeloma

We registered 6 patients with newly diagnosed multiple myeloma in 2012. A novel agent, the proteasome inhibitor bortezomib, became available in 2007, and we have used it with or without dexamethasone to treat patients who have refractory myeloma. A randomized phase II study was started in 2010 (JCOG0904) to evaluate the efficacy of bortezomib + dexamethasone versus thalidomide + dexamethasone in patients with relapsed or refractory chemoresistant multiple myeloma.

Hematopoietic stem cell transplantation

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

Solid tumors

Many patients with solid cancers have been referred to our division from related divisions or departments from both inside and outside our hospital. Several of our studies seeking improved therapeutic outcomes are in progress throughout our university hospital with related divisions or departments. The combination of fluorouracil (5-FU), epirubicin, and cyclophosphamide (FEC100) with or without taxotere therapy is an adjuvant therapy for patients with breast cancer treated with curative surgery. FEC100 followed by taxotere is a preoperative combination chemotherapy for patients with locally advanced breast cancer, aiming at limiting the extent of surgery. Adriamycin and taxotere followed by taxotere and trastuzumab is a first-line chemotherapy for patients with advanced, metastatic breast cancer. Since late 2008 we have been investigating a combined-modality therapy of radiation and chemotherapy with docetaxel, cisplatin, and 24 hours' continuous infusion of 5-FU (DCF regimen) for patients with locally advanced esophageal cancer. The study has been completed, and an improved protocol was launched in 2010. A novel drug-development study with an orally decaying formulation of S-1 in patients with advanced gastric cancer was finished in 2011. A multicenter cooperative randomized phase II study was started in 2011 to compare S-1 + cisplatin, S-1 + leucovorin, and S-1 + leucovorin + oxaliplatin for patients with advanced and recurrent gastric cancer and was completed in 2012. Because trastuzumab is also active in patients with human epidermal growth factor receptor 2-positive gastric cancer, we

treat such patients with capecitabine + cisplatin (XP) + trastuzumab. Our first-line chemotherapies for patients with advanced colorectal cancer are folinic acid, fluorouracil, and oxaliplatin (FOLFOX) and folinic acid, 5-FU, and irinotecan (FOLFIRI). Since antibodies against vascular endothelial growth factor and against epidermal growth factor receptor became available in 2007 and 2008, respectively, combination therapies of these antibodies and FOLFOX or FOLFIRI have also been performed.

Palliative care

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

Basic research

One of our important activities is translational research on solid cancers and hematological malignancies. Deep and durable remission in hematological malignancies is essential for longer survival and even cure. Exploring minimal residual disease can be useful for evaluating eradication of malignant cells. We are now preparing a minimal residual disease assay using bone marrow cells from patients treated for multiple myeloma by applying the real-time polymerase chain reaction.

Our studies of cancer fatigue are focused on patients with malignant disease. We are also preparing a study protocol evaluating fatigue by examining saliva in patients with multiple myeloma and breast cancer. By detecting human herpes simplex virus 6 particles in saliva, it would be possible to evaluate the degree of fatigue. Drug-sensitivity testing, to determine which patients are sensitive to which agent, is also important for cancer chemotherapy.

We are examining the correlation between drug sensitivity and gene expression, such as excision repair cross complementation group 1 and TS, using tissue samples from patients undergoing preoperative chemotherapy with docetaxel, cisplatin, and 5-FU for operable, locally advanced esophageal cancer.

Some of above mentioned research results have recently been collected for presentation at scientific meetings, and further research is in progress.

Publications

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Department of Internal Medicine

Division of Respiratory Diseases

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

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

General Summary

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

Research Activities

COPD

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

Infection and Lung injury

A double-stranded RNA virus is associated with acute lung injury. We investigated the effect of insulin on epithelial cell fate after damage by polyinosinic:polycytidylic acid. We used human bronchial epithelial primary culture cells and found that insulin was required to protect these cells from apoptosis induced by polyinosinic:polycytidylic acid. Apoptotic signals were dependent on caspase-8 activation. We also found that survival signals were transmitted mainly through activation of extracellular signal

regulated kinase and v-akt murine thymoma viral oncogene homolog, although other survival signals were also associated. We suggest that insulin is a promising treatment for acute lung injury induced by viral infection. These results were published in *Journal of Immunology*. We are extending this study to investigate the significance of innate immunity on lung injury induced by infection.

We investigated the usefulness of multiplex polymerase chain reaction for detecting the pathogens of pneumonia and infections that trigger the acute exacerbations of COPD and bronchial asthma. We found that the multiplex polymerase chain reaction was more sensitive than conventional tests.

Idiopathic pulmonary fibrosis

Aberrant re-epithelialization with bronchial epithelial cells is a prominent pathologic finding in idiopathic pulmonary fibrosis (IPF) and is implicated in abnormal epithelial-mesenchymal interactions. Recent studies have identified senescence as a risk factor for the development of IPF. Among the SIRT family of class III histone deacetylases (HDACs), SIRT6 has been shown to antagonize senescence. We examined epithelial senescence as a representative phenotypic alteration in conjunction with SIRT6 expression in IPF. We have found evidence that IPF lungs show enhanced senescence with a concomitant increase in SIRT6 expression in epithelial cells, including aberrantly re-epithelialized bronchial cells. We are also investigating the role of autophagy in IPF. We found that autophagy was accelerated in epithelial cells of IPF but that apoptosis and senescence overcome the protective effects of autophagy. These results were published in *American Journal of Physiology Lung Cell and Molecular Physiology*.

Autophagy on bronchiolar epithelial cells

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

In addition, we investigated mitophagy, which specifically targets damaged mitochondria and maintains the homeostasis of mitochondria biogenesis. Mitophagy was consistent with nonspecific autophagy and plays important roles in intracellular homeostasis. We are investigating the roles of mitophagy on the pathophysiology of COPD, IPF, and lung injury.

Lung cancer

Our clinical research on the effects of nitroglycerin on chemotherapy in non-small-cell lung cancer is continuing. 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. We started a clinical study of the effectiveness of TS-1 in elderly patients with non-small-cell lung cancer.

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Department of Internal Medicine

Division of General Medicine

Tatsuo Hosoya, *Professor*
 Iwao Ohno, *Professor*
 Masami Nemoto, *Associate Professor*
 Nobuyuki Furutani, *Associate Professor*
 Takanori Ebisawa, *Assistant Professor*

Norio Tada, *Professor*
 Hiroshi Yoshida, *Professor*
 Jun Hiramoto, *Associate Professor*
 Mie Kawai, *Assistant Professor*

Research Activities

Division of General Medicine, The Jikei University Hospital

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

Division of General Medicine, The Jikei University Katsushika Medical Center

We investigated the biological and molecular factors affecting the honeymoon period in Japanese patients with adult-onset type 1 diabetes. The insulin secretory capacity, lipid profile, and insulin dose requirement were identified as factors potentially affecting the occurrence of the honeymoon period. We presented several case reports associated with endocrine diseases: pituitary, adrenal, and parathyroid disease.

Division of General Medicine, The Jikei University Daisan Hospital

1. Study of nutritional support in elderly patients

We studied nutrition support in elderly patients. We found that the consumption of fewer calories and fluids improves outcomes and reduces the pain of elderly patients.

2. Study of polymyalgia rheumatica

We found that high levels of C-reactive protein and matrix metalloproteinase 3 at the start of steroid therapy and a slow decrease in matrix metalloproteinase 3 during therapy are associated with a high frequency of relapse. In such cases, we administer methotrexate, but improvement is difficult to achieve in many cases.

3. Study of hyponatremia in elderly patients

Hyponatremia is a frequent electrolyte disorder in elderly patients. We found that inflammation and mental stress readily cause the nonosmotic secretion of antidiuretic hormone and hyponatremia in elderly patients.

4. Study of procalcitonin

A high level of procalcitonin suggests sepsis-induced bacterial infection. However, we must remember that several noninfectious diseases causes cytokine storm and high levels of procalcitonin.

Division of General Medicine, The Jikei University Kashiwa Hospital

Our research works consist of 2 parts. The first is to investigate the role of general medicine on environmental health achievements in regional areas, especially in Kashiwa City. The second part is to develop educational tasks for teaching medical students and junior physicians.

Publications

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Department of Psychiatry

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

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

Research Activities

Psychopathology, psychotherapy and child study group

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

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

Morita therapy group

We have been continuously promoting comparative studies between Morita therapy and the third generation of cognitive-behavioral therapies, such as acceptance and commitment therapy and mindfulness-based cognitive therapy, and the results have been reported at several conferences. Recently, we have applied Morita therapy to traumatic stress or posttraumatic stress disorder, pain disorders, and emetophobia and reported clinical studies of these topics. In addition, studies continuing this year examined the subtypes of obsessive-compulsive disorder, the psychopathology of social anxiety disorders, and factors in the recovery from depression with inpatient Morita therapy.

Psychopharmacology group

In basic research, we performed the following studies in rodents: 1) effect of a novel psychotropic agent on monoamine neurotransmission using microdialysis and radioimmunoassay techniques, 2) the formation mechanism of drug addiction, 3) the neural basis of

addiction-related impulsivity, and 4) the development of a novel anticraving agent (the latter 3 studies were performed in collaboration with the NTT Communication Science Laboratories and the Department of Psychology, Senshu University). In clinical research, we have performed the following studies in humans: 1) the effect of second-generation antipsychotics on anxiety and stress-related disorders, 2) the effect of antipsychotics on dopaminergic neurotransmission using positron emission tomography (in collaboration with the National Institute of Radiological Sciences), 3) exploratory research on novel blood biomarkers for mood disorders (in collaboration with the Department of Virology, The Jikei University School of Medicine), and 4) the effect of modified electroconvulsive therapy on regulatory factors for gene expression, and 5) the symptoms of menstruation-related mental disorders, atypical psychosis, and acute psychosis. Integration between basic and clinical research is a fundamental concept of the Psychopharmacology group.

Psychophysiology group

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

Psychogeriatric group

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

General hospital psychiatry

In a study of interventional therapy based on cognitive-behavioral therapy aimed at preventing recurrences of depression, a computer system and sleep evaluation methods were introduced to a previous evaluation system for more effective presentations and for more precise estimation, respectively. Furthermore, an investigation of new indications for this intervention for patients with atypical depression, bipolar depression, and insomnia was performed. Another study investigated the issues associated with mental care services for patients with cancer. We are focusing on risk factors for postoperative delirium in patients with digestive tract cancers.

Clinical electroencephalography group

We reported a case of epilepsy in a patient with ring chromosome 20 syndrome, and from

a review of the literature, we discussed the characteristics of patients with ring chromosome 20 syndrome. Furthermore, we reported a case of elderly-onset partial epilepsy with manic state due to postictal psychosis. A study was performed to prevent the recurrence of depression in patients with epilepsy. We are planning a study of the management of epilepsy in pregnant women.

Clinical psychology group

We have continued to discuss and study psychotherapeutic processes and the treatment techniques of cognitive behavior therapy, Morita therapy, relief care, psycho-oncology, and mental care after natural disasters. We have also examined the characteristics of developmental disorders and higher brain dysfunctions through psychological assessments. We invited Mr. Shu Morioka to a clinical conference and studied “social brain” in neuroscience. Furthermore, we trained graduate students of a clinical psychological course.

Publications

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Department of Pediatrics

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

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

General Summary

We have 10 subspecialty research groups, which are the Inherited Metabolic Disease group, the Endocrinology group, the Neurology group, the Hematology and Oncology group, the Infectious Diseases and Immunologic Disorders group, the Nephrology group, the Cardiology group, the Allergy group, the Neonatology group, and the Pediatric Psychiatry group. The final aim of each subspecialty groups is supplying practical benefits to patients and their families through basic and translational research and clinical study.

Research Activities

Inherited metabolic disease group

1. We developed a novel gene therapy for Krabbe disease and mucopolysaccharidosis (MPS) type VII.
2. We showed the effectiveness of preconditioning, such as with interferon and an anti-c-kit antibody, for bone marrow transplantation in a murine model of MPS type II.
3. We found that enzyme replacement therapy has an additive effect to bone marrow transplantation in MPS II mice.
4. Induction of a molecular chaperone enhanced the activity of α -glucosidase in Pompe disease fibroblasts.
5. We showed that oral administration of α -glucosidase induced immune tolerance in enzyme replacement therapy for Pompe disease.
6. A database was created of patients in our clinic with Fabry disease.
7. We performed genetic diagnoses with a comparative genomic hybridization array and next-generation sequencing in patients with congenital anomalies and intellectual disabilities.

Neurology group

We investigated the clinical records of 142 children after traumatic brain injury to evaluate the outcomes of posttraumatic epilepsy. The risk factors for post-traumatic epilepsy were abuse, subdural hematoma, consciousness disturbance (severe, prolonged), and complications (poor mobility, cognitive impairment). We evaluated the efficacy of fosphenytoin in 24 patients with status epilepticus. The

efficacy of fosphenytoin was 70%. Transient hypotension was observed as an adverse effect in 1 patient. The optimal serum concentration of fosphenytoin could be maintained for 15 hours with a dose of 22.5 mg/kg but could not be achieved with a dose of 7.5 mg/kg. Our findings suggest that fosphenytoin is safe and effective for patients with status epilepticus who are younger than 2 years.

Nephrology group

The Division of Nephrology provides a full range of services for the evaluation and management of children with simple or complex nephrologic or urologic disorders, including:

1. Hematuria or proteinuria or both
2. Glomerulonephritis
3. Urinary tract infections
4. Electrolyte or acid-base disorders
5. Hypertension
6. Complex and severe disorders resulting in end-stage renal disease requiring dialysis or transplantation or both

Our staff is well equipped to provide both acute and chronic management of various kidney problems and to provide consultative services to other departments within the hospital and to community physicians. Inpatient and outpatient consultations are available for children with electrolyte and acid-base disorders. Staff members work closely with urologic and pediatric surgeons to provide comprehensive management of patients.

Infectious diseases and Immunologic Disorders group

We focused on the identification of causative pathogens by means of polymerase chain reaction techniques for the genetic diagnosis and treatment of primary immunodeficiency syndrome, especially chronic granulomatous disease (CGD). The multiplex polymerase chain reaction assay was faster, more sensitive, and more specific than the gold-standard culture-based method in inflammatory diseases and respiratory infectious diseases.

We found a significantly lower incidences of *Bacteroides* and *Bifidobacterium* in patients with CGD and colitis than in patients with CGD and no colitis. The results suggest that compositional change is a useful diagnostic tool in CGD colitis.

We reported that thalidomide was effective for treating bowel inflammation in patients with CGD but did not cause progression of fungal or bacterial infections. Thalidomide is an efficacious therapeutic option for patients with CGD colitis.

Hematology and Oncology group

We reported familial cases of *MYH9* disorders. The patients were a 1-year-old Japanese boy, who presented only with macrothrombocytopenia, and his father, who had refractory chronic idiopathic thrombocytopenia purpura, hearing loss and, chronic renal failure. Sequence analysis of exon 1 of the *MYH9* gene identified heterozygous S96L mutations in both the child and the father, resulting in the diagnosis of familial cases of *MYH9* disorders.

We performed and presented a cross-sectional survey of pain management during bone marrow aspiration and biopsy to institutions belonging to the Tokyo Children's Cancer Study Group. Through the acquisition of relevant pharmacological knowledge and the sharing of information with palliative care teams, the "Guide to the Pharmacological Management of Symptoms in Children with Cancer" was established.

Cardiology group

The pediatric cardiology group is interested in both basic and clinical cardiology research to improve outcomes for children with congenital or acquired heart diseases. The results of our research have been presented at the annual meetings of the Japan Pediatric Society, the Japan Pediatric Cardiology and Cardiac Surgery, and the Pediatric Academy Society. Ongoing projects are as follows.

1. The effects of telmisaltan in heart failure
2. The effects of bisoprolol in right heart failure
3. The effects of carperitide in monocrotaline-induced pulmonary hypertension
4. Assessment of cardiopulmonary function in metabolic heart disease
5. Urocortin and angiotensin evaluation in congenital heart disease
6. Early diagnosis of renal dysfunction in patients with congenital heart disease
7. Risk factors for atrial tachycardia in Wolff-Parkinson-White syndrome

Allergy group

The main subjects of our research are as follows: 1) the role of eosinophils, mast cells, and epithelial cells in the pathology of allergic diseases; 2) pediatric asthma; 3) food allergy; 4) atopic dermatitis; and 5) treatments for allergic diseases. We have organized and performed the following multicenter clinical studies: 1) The Preventive Effect of Tulobuterol Patch for the Long-Term Management of Infantile Asthma study (PET study); 2) Pediatric Asthma Research for Guideline study (Pediatric Asthma Research for Guideline Update: Add-on use of tulobuterol patch on unstable asthma treated with leukotriene receptor antagonist); 3) The Continuous Inhalation Treatment study (A comparison of continuous inhalation treatment with salbutamol and isoproterenol for severe pediatric bronchial asthma: A multicenter, double-blind, randomized study); and 4) the Optimal Stepdown Therapy for Controlled Pediatric Asthma Responded to SFC (OSCAR) study; Effect of Oral Immunotherapy in Preschool Children with Milk Allergy study (ORIMA study).

Endocrinology group

Accomplishments of our group this year are as follows.

1. We analyzed the expression of urocortin 2, urocortin 3, corticotropin-releasing factor receptor 2, nesfatin-1, and inflammatory cytokines in the brains of a rat model of left ventricular heart strain. Furthermore, we are performing immunohistochemical and behavior analyses.
2. We studied insulin insufficiency in infants with acute gastroenteritis due to *Rotavirus* infection.
3. We investigated the efficacy of treatment with growth hormone in children who are

small for gestational age, have short stature, or had extremely low birth weight. Both good responders and poor responders to growth hormone treatment were clearly identified.

4. We have just started an educational program for young pediatric endocrinologists. We are now sending each young resident to the Departments of Endocrinology and Metabolism of Saitama Children's Medical Center and of Tokyo Metropolitan Children's Medical Center.

Publications

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Department of Dermatology

Hidemi Nakagawa, *Professor*
Mariko Honda, *Professor*
Arihito Ota, *Associate Professor*
Masaaki Kawase, *Associate Professor*
Yoshinori Umezawa, *Assistant Professor*
Yoshimasa Nobeyama, *Assistant Professor*

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

General Summary

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

Research Activities

Psoriasis

Various systemic therapies, including oral cyclosporin microemulsion preconcentrate, methotrexate, etretinate and topical vitamin D3, and corticosteroids, have been used, depending on disease severity and the degree to which quality of life (QOL) is impaired in individual patients. Also phototherapy, including psoralen ultraviolet A, narrow-band ultraviolet B (UVB), and the 308-nm excimer lamp, are effective and have been administered in a newly organized skin-care clinic. We have evaluated patients' QOL reflecting social background and have developed a Japanese version of the Psoriasis Disability Index. We also developed a Japanese version of the Work Productivity and Activity Impairment questionnaire for psoriasis. We examined the incidence of metabolic syndromes as a comorbidity of psoriasis. In a special psoriasis clinic, we select patient-based treatments to satisfy patients' demands. New biologic agents, including infliximab, adalimumab, and ustekinumab, are available and have been used to treat intractable psoriasis. Clinical trials have been performed with new biologic agents, including antibodies against interleukin (IL)-17A, IL-17 receptor, IL-23p19, and janus kinase 1/3 inhibitor.

Atopic dermatitis

The pathogenesis of atopic dermatitis has been attributed to a complex interaction of the environment, host susceptibility genes, altered skin barrier function, and the immune system. Recently, psychosocial factors have been suggested to influence the exacerbation of atopic dermatitis. Therefore, we are treating patients on the basis of both evidence-based medicine and QOL issues. We try to obtain a precise medical history from each patient and to evaluate how QOL is impaired. We have evaluated the patients' sleep quality using the Pittsburgh Sleep Quality Index and found that its score was positively associated with the scores of the Severity Scoring of Atopic Dermatitis Index and the

Dermatology Life Quality Index, indicating that nocturnal itching and scratching behavior impair the sleep quality of patients with atopic dermatitis.

In basic clinical research, the levels of substance P, thymus and activation-regulated chemokine, and IL-31 related to pruritus in atopic dermatitis are being evaluated according to disease severity. Clinical trials of topical phosphodiesterase 4 inhibitor have been performed.

Malignant skin tumors

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

Neurofibromatosis

Because the number of registered patients in our clinic is the largest in Japan and many patients with letters of introduction visit from all over Japan, we concentrate on long-term follow-up and improvement of impaired QOL by means of accurate diagnosis and the resection of neurofibromas. The estimated lifetime risk of MPNST in patients with neurofibromatosis 1 is 10%, although information concerning the epigenetic abnormality is limited. We have used the methylation-specific polymerase chain reaction (PCR) and real-time reverse-transcriptase-PCR to analyze the methylation status of tumor suppressor genes and cancer-testis genes in established MPNST cell lines. The findings of abnormal expression of several cancer-testis genes and the inactivation of tumor-suppressor genes indicate that disarranged methylation and demethylation are involved in the ontogenesis of MPNST.

Herpes virus infection

1. Herpes simplex virus

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

2. Herpes zoster and postherpetic neuralgia

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

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

Human papillomavirus infection

In addition to ordinary cryotherapy, treatments for viral warts include topical vitamin D3, salicylic acid, glutaraldehyde, and monochloroacetic acid. Contact immunotherapy with squaric acid dibutylester, CO₂ laser, and pulsed dye laser have also been used to treat severe, intractable viral warts. Human papillomavirus infection typing with the PCR has regularly been performed for bowenoid papulosis and rare viral warts. Five percent imiquimod cream is now available for the treatment of condyloma acuminatum.

Contact dermatitis/drug eruption

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

Laser

The Q-switched ruby laser is useful for treating nevus Ota, acquired dermal melanocytosis, and ectopic Mongolian spot because of its selective photothermolysis. Such treatment is covered by health insurance. Senile freckles are usually successfully treated with a single treatment, but treatment is not covered by health insurance, so is performed at the patient's personal expense. On the other hand, nevus spilus is difficult to treat with the Q-switched ruby laser because it often recurs after 1 to 2 months. The efficacy of a pulsed dye laser for treating hemangiomas and telangiectasia depends on the clinical type, location, patient age, and other factors. The pulsed dye laser was effective for treating hemangioma simplex on the face or neck of young adults. The size and redness of the strawberry mark can be reduced if treatment is started before the age of 6 months. The recently introduced V-beam laser is effective for intractable vascular lesions. We have been able to use the V-beam laser since 2011. Because the ultrapulse CO₂ laser has higher energy and a shorter pulse width, it can vaporize at a fixed depth and can be used to quickly remove actinic keratosis, seborrheic keratosis, syringoma, and epidermal nevus.

Skin Care Clinic

Narrow-band UVB irradiation is performed for patients with psoriasis, alopecia, atopic

dermatitis, prurigo nodularis, vitiligo, or cutaneous T-cell lymphomas. Targeted phototherapy equipment, such as the 308-nm excimer light, is also used. Other special clinics, including those for skin care lessons, therapeutic make-up, acne care, mental care, and *kampo* medicine, are available to patients on demand.

Self-assessment

Psoriasis: To improve patients' QOL and treatment compliance, we have selected therapies on the basis of their risk/benefit ratios. Phototherapy with narrow-band UVB and the 308-nm excimer lamp has been introduced. Biologic agents, including infliximab, adalimumab, and ustekinumab, have also been used to treat patients with severe psoriasis. **Neurofibromatosis:** Many patients with neurofibromatosis type I are still being referred to our special clinic. We are now performing inheritance consultation for pediatric patients. Surgical removal of different types of neurofibroma is performed for inpatients and outpatients to enhance QOL. Genetic analysis has been performed for MPNST.

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

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

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

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

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

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

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

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Department of Radiology

Kunihiko Fukuda, *Professor*
 Junta Harada, *Professor*
 Yukio Miyamoto, *Professor*
 Shunichi Sadaoka, *Associate Professor*
 Hiroya Ojiri, *Associate Professor*
 Norio Nakata, *Associate Professor*
 Masao Kobayashi, *Assistant Professor*

Chihiro Kanehira, *Professor*
 Toru Sekiya, *Professor*
 Hiroshi Sekine, *Professor*
 Mayuki Uchiyama, *Associate Professor*
 Manabu Aoki, *Associate Professor*
 Yoshimitsu Sunagawa, *Assistant Professor*
 Takuji Mogami, *Assistant Professor*

Research Activities

Division of diagnostic imaging

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

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

2. CT of eosinophilic chronic rhinosinusitis

Eosinophilic chronic rhinosinusitis is a newly recognized subtype of chronic rhinosinusitis, which is characterized by peripheral blood eosinophilia and massive infiltration of eosinophils in the nasal mucosa. We proposed CT diagnostic criteria for eosinophilic chronic rhinosinusitis and analyzed their clinical usefulness.

3. The value of specific magnetic resonance features in the evaluation of suspected placental invasion

We evaluated magnetic resonance (MR) imaging findings that may help predict the presence of placenta accreta, placenta increta, or placenta percreta. MR can be a useful adjunct to ultrasound in diagnosing placenta accreta prenatally. Three features that are seen on MR in patients with placental invasion appear to be useful for diagnosis: uterine bulging, abnormal placental flow voids, and the presence of dark intraplacental bands on T2-weighted imaging.

4. Imaging features of primary fallopian tube cancer, with a focus on diffusion-weighted images and contrast-enhancement patterns

We reviewed MR and CT findings of 6 patients with pathologically diagnosed primary fallopian tube cancers. These cancers are small, solid, malignant tumors that are often sausage-shaped and show restricted diffusion, early enhancement, and rim enhancement. Both MR and CT play important roles in the detection and staging of primary fallopian tube cancers.

5. Tomosynthesis to detect fragility fractures of the pelvis

The aim of this study was to evaluate the diagnostic performance of tomosynthesis in visualizing fragility fractures of the pelvic bone, with the use of MR and CT as references, and to determine whether tomosynthesis is more effective than X-rays for detecting these lesions.

6. Morphological and hemodynamic evaluation of the cardiovascular system with dual-

source CT

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

7. Imaging ovarian borderline tumors

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

8. Usefulness of contrast-enhanced MR for evaluating the therapeutic effects of biological agents against tumor necrosis factor α for psoriatic arthropathy

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

9. Evaluation of bone marrow signal abnormalities at cruciate ligament entheses

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

Division of Ultrasound

1. Clinical usefulness of sonographic contrast examination of breast tumors

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

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

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

Division of Nuclear Medicine

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

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

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

Division of Interventional Radiology

1. Efficacy and safety of our new technique of ipsilateral percutaneous transhepatic portal vein embolization

Percutaneous transhepatic portal vein embolization was performed to increase the volume of the left hepatic lobe before hepatic resection in 8 patients. With ultrasonic guidance, a balloon catheter was introduced into the right portal vein. A gelatin sponge was injected via the sheath while the right portal vein was occluded with a balloon. Two weeks after the procedure the volume of the left hepatic lobe was assessed with either CT or scintigraphy. The volume of the future liver remnant was increased by $46.5\% \pm 31.5\%$. There was no complications or progressive liver insufficiency after embolization or resection.

Division of Radiation Therapy

1. Clinical study of radiosensitization therapy via topical injection of low-concentration hydrogen peroxide and hyaluronate

Radioresistant tumors are hypoxic and have increased peroxidase activity. Hydrogen peroxide and hyaluronate are injected topically at low concentrations and are then degraded to produce oxygen. Tumor tissue can be re-oxygenated by inactivating peroxidase/catarase. In this way, low-linear energy transfer radioresistant tumors can be transformed into radiosensitive tumors. The purpose of this study was to confirm the local enhancement effect of radiotherapy with this method, which was established by Kochi University as Kochi Oxydol-Radiation Therapy for Unresectable Carcinomas (KOR-

TUC).

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

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

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

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Department of Surgery

Division of Digestive Surgery

Katsuhiko Yanaga, *Professor*
 Nobuyoshi Hanyu, *Visiting Professor*
 Tetsuji Fujita, *Associate Professor*
 Tomoyoshi Okamoto, *Associate Professor*
 Noburo Omura, *Associate Professor*
 Satoru Yanagisawa, *Associate Professor*
 Yuichi Ishida, *Associate Professor*
 Kouji Nakada, *Assistant Professor*
 Yoichi Toyama, *Assistant Professor*
 Yoshiyuki Hoya, *Assistant Professor*
 Masaichi Ogawa, *Assistant Professor*
 Shigeki Wakiyama, *Assistant Professor*
 Shuichi Fujioka, *Assistant Professor*
 Fumiaki Yano, *Assistant Professor*

Kazuhiko Yoshida, *Professor*
 Hideyuki Kashiwagi, *Visiting Professor*
 Norio Mitsumori, *Associate Professor*
 Takeyuki Misawa, *Associate Professor*
 Kazuo Matai, *Associate Professor*
 Hidejiro Kawahara, *Associate Professor*
 Yuji Ishii, *Assistant Professor*
 Shuzo Kono, *Assistant Professor*
 Yoshio Ishibashi, *Assistant Professor*
 Naoto Takahashi, *Assistant Professor*
 Katsunori Nishikawa, *Assistant Professor*
 Ken Eto, *Assistant Professor*
 Yasuro Futagawa, *Assistant Professor*

General Summary

Research activities in clinical medicine should be measured not by the number of abstracts accepted at scientific meetings and publications in the journals with a low impact factor, but by the number of publications in core clinical journals. Such journals in the field of digestive and general surgery include *Annals of Surgery*, *British Journal of Surgery*, *Journal of the American College of Surgeons*, *JAMA Surgery* (previously *Archives of Surgery*), *Surgery, American Journal of Surgery*, and other subspecialty journals, such as *Transplantation*. In addition to successfully performing a study acceptable for publication in these journals, we must avoid scientific misconduct that could cause confusion in the field of surgical science. According to a recent article in *European Heart Journal*, William T. Summerlin, a transplant immunologist claimed in 1971 that rejection in skin grafts could be avoided, if the material is treated with a form of tissue culture before transplantation. This was a discovery that brought him a position at the Sloan-Kettering Institute in New York as the chief of transplantation immunology. Unfortunately, in New York, he could not replicate his previous experimental results — obviously this can happen, as any scientist knows. In desperation, he colored some of the grafts of his white mice with a black felt-tip pen. This misconduct was immediately discovered, and during the inquiry, doubts also fell on his previous work. Scientific reports are based on empirical observation, precise wording, consistent statements about facts, and their interaction supported by appropriate statistics. Researchers should be familiar with medical statistics, and all authors should carefully read and approve the manuscripts they are involved with.

Research Activities

Upper gastrointestinal surgery

We began to use high-resolution manometry and multichannel intraluminal impedance

pH monitoring to study the pathogenesis of primary esophageal motor functional disorders, such as achalasia and gastroesophageal reflux disease. We investigated the significance of the expression of small ubiquitin-like modifier (SUMO) 1 as a prognostic factor in esophageal cancer. We found that overexpression of SUMO-1 correlated with malignancy-associated pathological findings and a poor prognosis. We continue to assess the viability of the gastric tube during esophagectomy using an intraoperative thermal imaging system; our findings suggest a correlation between optimal graft construction and postoperative complications. The sentinel lymph node navigation system may play a key role in limited surgery for early gastric cancer.

In 553 patients who underwent laparoscopic gastrectomy, gastric cancer recurred in 5 (0.9%) patients (3 patients with stage I disease, 1 patient with stage II disease, and 1 with stage III disease). Postgastrectomy syndrome comprises specific symptoms after gastrectomy and is a target for treatment. To decrease the incidence and severity of postgastrectomy syndrome and to maximize residual gastric function, several types of limited gastric resection with refined techniques of reconstruction have been attempted. In addition, multiple tests of postoperative gastrointestinal function are applied to patients who have undergone gastrectomy to evaluate various gastrectomy procedures and to inform the patients of the advantages and disadvantages of each procedure.

Lower gastrointestinal surgery

We are using a virtual reality surgical simulator to train surgical residents. We compared surgeons' stress between laparoscopic surgery and open surgery by measuring the serum levels of epinephrine, norepinephrine, dopamine, adrenocorticotrophic hormone, and cortisol. We are analyzing the data to determine whether the measurement of such variables might contribute to the training of the next generation of laparoscopic surgeons.

We are preparing a collaborative study with the Department of Urology to use proteomics to identify novel cancer-related proteins in gastrointestinal cancers (including colon, esophageal, gastric, pancreatic, and liver cancers). The aim of this study is to identify tumor markers or possible target proteins via comprehensive protein analysis of biopsy specimens from cancerous tissue and noncancerous mucosa.

The relationships of copy number variation to recurrence and prognosis are evaluated after DNA is extracted from frozen specimens of colorectal cancer tissue, which have been stored in our department. For genes showing copy number variation, the intracellular gene expression varies significantly. This variable expression, therefore, may affect gene functions. In collaboration with the Department of Biochemistry, we have committed to constructing a complementary DNA library from surgical specimens of colorectal cancer to analyze the expression of intracellular signal molecules associated with the progression and growth of colorectal cancers. As a first step of this project, we have started to analyze the cell-cycle regulation and dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 in relation to c-Jun/c-Myc phosphorylation. In addition to these analyses, we will strengthen a platform for our future basic research through a complementary DNA library and a clinical database.

Hepatobiliary and pancreatic surgery

Our main research activities are as follows.

1. Living donor liver transplantation (LDLT) and regenerative medicine
2. Treatment for hepatocellular carcinoma (HCC) and control of recurrence
3. Chemotherapy for pancreatic and biliary cancer
4. Expansion of surgical indications for multiple hepatic tumors
5. Laparoscopic surgery for the liver, biliary tree, pancreas, and spleen
6. Navigation surgery for hepatobiliary and pancreatic diseases
7. Nutritional therapy for cancer patients
8. Control of surgical site infection in surgical patients
9. Effect of preoperative treatment of eltrombopag on splenectomy for idiopathic thrombocytopenic purpura
10. Genome-wide association study for donors and recipients in LDLT
11. Molecularly targeted therapy for advanced HCC
12. Analyses of new biological tumor markers for HCC

The first LDLT was successfully performed for a patient with postnecrotic cirrhosis and HCC on February 9, 2007. Our 13th LDLT was performed for a patient with primary biliary cirrhosis on May 31, 2013. All 13 recipients were discharged in good condition from postoperative days 15 to 55, and all donors were discharged on postoperative days 8 to 26 and have returned to their preoperative status. We are planning to extend the indications for LDLT to ABO blood type-incompatible patients and patients with acute hepatic failure. We have performed translational research for the combination chemotherapy of gemcitabine and a new protease inhibitor, FUT-175, which is associated with both nuclear factor κ -B inhibition and apoptosis induction in pancreatic cancer cell lines. We have started treatment with a new 3-drug combination chemotherapy comprising gemcitabine, TS-1, and FUT-175 for advanced pancreatic cancer. Navigation for liver resection is covered by the national health insurance system as of April 1, 2012, and the Vincent navigation system was introduced in July 2012. Biliary and pancreatic navigation surgery is performed with the Institute for High Dimensional Medical Imaging Research Center. Other clinical and experimental trials are ongoing regarding treatment of hepatic tumors and of laparoscopic surgery, nutritional therapy, surgical site infection, and eltrombopag as a pretreatment for laparoscopic splenectomy in cases of idiopathic thrombocytopenic purpura. Also, we are participating in multicenter studies of genome-wide associations, molecularly targeted therapy for advanced HCC, and new biological tumor markers for HCC.

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Department of Surgery

Division of Chest Surgery, Breast and Endocrinology Surgery

Toshiaki Morikawa, *Professor*
Tadashi Akiba, *Professor*
Shuji Sato, *Assistant Professor*
Makoto Odaka, *Assistant Professor*

Hiroshi Takeyama, *Associate Professor*
Satoki Kinoshita, *Associate Professor*
Yasuo Toriumi, *Associate Professor*
Kazumi Kawase, *Assistant Professor*
Isao Tabei, *Assistant Professor*
Hiroko Nogi, *Assistant Professor*

General Summary

The Divisions of Chest Surgery and of Breast and Endocrinology Surgery were established in June 2005. Since then, all staff members have been active in surgical practice, research, and education. Many studies are 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, in addition to resection of mediastinal tumors or the thymus. Surgery for lung cancer requires much more advanced skills and oncological considerations, which have also been independently developed. Of the mediastinal procedures, thymectomy is usually performed via thoracoscopy rather than via a conventional median sternotomy. In our department the percentage of the chest operations performed via thoracoscopy is more than 90%, which we assume to be the highest rate in the world.

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

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

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

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

Breast

1. Clinical study

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

The disease status of the axillary lymph nodes is the most important prognostic factor for breast cancer. However, axillary lymph-node dissection is associated with postoperative morbidities, such as upper extremity edema, pain, paresthesia, and restriction of the shoulder girdle. The minimally invasive technique of sentinel lymph-node biopsy produces less morbidity and yet allows accurate pathologic staging of the axilla. Experience with sentinel lymph-node biopsy after neoadjuvant chemotherapy is limited. The purpose of our clinical study is to evaluate the feasibility, accuracy, and safety of this procedure in patients who have breast cancer treated with neoadjuvant chemotherapy.

2) The evaluation of Sonazoid

We performed phase II and III studies of ultrasonographic imaging of the breast with the microbubble contrast medium Sonazoid (Daiichi Sankyo Co., Ltd., Tokyo) in collaboration with the Department of Radiology. With Sonazoid the sensitivity of ultrasonography for detecting small cancers of the breast equals that of magnetic resonance imaging.

3) The evaluation of psychiatric illnesses of patients with breast cancer

For some patients with breast cancer, psychiatric illnesses, such as depression, are associated with the development of breast cancer. We have analyzed patients with breast cancer who have undergone surgery.

2. Basic research

1) Studies of the early development of breast cancer

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

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

Triple-negative breast cancer (TNBC) tumors do not express estrogen receptor or progesterone receptor and do not overexpress human epidermal growth factor receptor 2. His-

torically, TNBC has responded well in the neoadjuvant setting, with rates of pathologic complete response commonly higher than for other types of breast tumor. However, more than half of patients with TNBC do not achieve a pathologic complete response and have a very poor prognosis. Gene-expression profiling demonstrated that TNBC is a highly heterogeneous disease, including 2 basal-like, immunomodulatory, mesenchymal, mesenchymal stem-like, and luminal androgen receptor subtypes. By analyzing biological markers, we have attempted to identify chemosensitivity factors in TNBC.

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

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

Endocrine

1. Basic research

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

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

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

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

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Department of Surgery

Division of Pediatric Surgery and Vascular Surgery

Takao Ohki, *Professor and Chairperson*
 Hitoshi Sakuda, *Assistant Professor*
 Naoki Toya, *Assistant Professor*

Atsushi Ishida, *Assistant Professor*
 Yuji Kanaoka, *Assistant Professor*
 Joji Yoshizawa, *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, treatment of peripheral arterial disease with drug-eluting stents, and clinical studies of specific antibodies for heparin-platelet factor 4 (PF4) complexes.

Research Activities

Pediatric Surgery

1. Education

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

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

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

2. Clinical studies

a. Endoscopic treatment for vesicoureteral reflux using Deflux®

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

b. Electrolyte and acid-base balances in laparoscopic surgery

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

c. In severe cases of gastroesophageal reflux, a surgical procedure called fundoplication is performed. This procedure is performed laparoscopically in our hospital. With minimally invasive laparoscopic surgery, pain is minimized, and the postoperative recovery time is shorter. The number of neurologically handicapped children treated at our hospi-

tal for gastroesophageal reflux has been increasing.

d. The Nuss procedure for treating pectus excavatum aims to force the sternum forward and hold it there with an implanted steel bar without requiring a large incision to resect the abnormal cartilage. In this procedure, the curved steel bar is placed under the sternum through 2 small incisions on the sides of the chest. The number of patients with pectus excavatum treated surgically in our department is the third highest in Japan.

3. Basic studies

a. Laparoscopic surgery contributes to global warming

Carbon dioxide, the most important greenhouse gas, is indispensable for laparoscopic surgery. To assess CO₂ emissions, we first determined the number of laparoscopic operations performed in Japan. Next, we measured the quantity of CO₂ used in our hospital.

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

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

c. Plasmapheresis in severe sepsis or septic shock

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

Vascular Surgery

1. Development of endovascular repair of thoracoabdominal aneurysms

Although stent grafts for the treatment of abdominal aortic aneurysms (AAAs) have been developed and are commercially available, no such stent grafts are available for the treatment of thoracoabdominal aortic aneurysms (TAAAs). The surgical mortality rate following open surgery for the treatment of AAAs is satisfactory, but that for TAAAs remains unacceptably high at 15% to 20%, and further improvement is desperately needed. Because a TAAA involves 1 or more visceral arteries, visceral perfusion must be maintained while the aneurysm is excluded with stent grafts. We have used a custom-made branched stent graft in combination with covered stents (for visceral reconstruction) for the treatment of TAAAs that were considered inoperable because of comorbid conditions or a hostile thorax/abdomen. Although stent graft repair for TAAAs requires long operative and fluoroscopic times, this treatment is feasible and safe.

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

We have developed a new minimally invasive operation for aortic arch aneurysms. After carotid-carotid bypass surgery is performed and stent grafts are placed, a needle is used to push the stent graft thorough one side of a carotid artery, after which a covered stent is inserted as a branch and deployed into the stent graft (in an in-situ retrograde fashion). We have examined this retrograde in-situ branched surgery in an *in-vitro* study and have applied it clinically. This operation is expected to be a less invasive surgery for aortic arch aneurysms.

3. Research on drug-eluting stents in the superficial femoral artery

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

Both a global registry and a randomized controlled trial, in which most patients were enrolled in the United States, but also in Germany and Japan, is awaiting its 1-year primary endpoint, which was reached in August 2009. We participated in this trial.

4. Clinical study of specific antibodies against heparin-PF4 complexes

Heparin is commonly used for anticoagulation in vascular surgery. Heparin-induced thrombocytopenia (HIT) is a rare but life-threatening complication with thrombosis of veins and arteries. Even if heparin use is limited, it occasionally induces the production of specific antibodies against heparin-PF4 complexes. Patients with such antibodies are at increased risk for HIT. The prevalence of these antibodies in patients receiving heparin is presumably underestimated. Accordingly, we prospectively measured antibodies against heparin-PF4 complexes and activity of PF4 and investigated whether they are related to symptoms of HIT, particularly in patients undergoing major vascular surgery. We measured these variables in 300 patients for 2 years.

The percentage of patients with antibodies to heparin-PF4 complexes was approximately 13%, which was higher than expected. Moreover, PF4 activity tended to be higher in antibody-positive patients than in antibody-negative patients. The results of this study are being statistically analyzed and were reported in 2009.

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

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

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Department of Orthopaedic Surgery

Keishi Marumo, *Professor*
 Takuya Otani, *Associate Professor*
 Shigeru Soshi, *Associate Professor*
 Makoto Kubota, *Assistant Professor*
 Yutaka Ueno, *Assistant Professor*

Hajime Sugiyama, *Associate Professor*
 Hiroki Funasaki, *Associate Professor*
 Mitsuru Saito, *Associate Professor*
 Mamoru Yoshida, *Assistant Professor*
 Hideki Fujii, *Assistant Professor*

General Summary

Basic Research

Our studies of bone metabolism and osteogenesis have been highly acclaimed both in Japan and abroad. The research on bone metabolism has been focused on the relationship between osteoporosis and fracture risk: detailed studies have been performed to understand in which cases fractures occur and what pathological processes lead to weakness of the osteoporotic bone. Our studies of β -tricalcium phosphate (β -TCP) have played a pioneering role in the field of bone grafting. They led to the wide application of β -TCP in many clinical settings; e.g., due to its efficient bone formation profile, β -TCP has been used as a complementary filling material in repairs of bone defects. Furthermore, studies of the relationship between micropores and osteogenic factors, such as bone morphogenic proteins (BMPs), facilitated further understanding of the osteogenesis mechanism.

Clinical Research

Our clinical practice has been divided into 8 subspecialties to treat a wide range of musculoskeletal disorders and is managed by different specialist teams: knee joint, hip joint, spine, shoulder joint, hand surgery, foot surgery, trauma, osteoporosis, and rheumatic diseases. All teams maintain a high level of expertise and are actively involved in scientific activities. The spine team has demonstrated the effectiveness of minimally invasive spine stabilization with percutaneous pedicle screw fixation in the treatment of infectious spondylitis. The hip joint team has proven the importance of the obturator externus muscle in soft-tissue repair during posterior-approach total hip arthroplasty and obtained good outcomes by applying the surgical method they developed. The knee joint team has been performing total knee arthroplasties with patient-matched instrumentation and has analyzed the effectiveness of cutting-edge technologies even more advanced than the surgical navigation system itself. Through this wide range of clinical research activities, all teams fulfill their important roles at a clinical academic hospital, and their commitment has been highly evaluated.

Research Activities

Treatment methods for complete dislocation of the acromioclavicular joint

We reviewed results of the modified Cadenat procedure, surgery with a poly-L-lactic acid ligament augmentation device (PLLAD), and nonoperative treatment for grade III dislo-

cations of the acromioclavicular joint. There were 63, 10, and 30 patients in the respective groups, and they were followed up for an average of 27, 28, and 11 months, respectively. At final follow-up, the average JOA score was 93 points in patients who underwent the modified Cadenat procedure, 92 points in patients treated with the PLLAD device, and 94 points in patients who underwent nonoperative treatment. Two patients who underwent nonoperative treatment requested surgical treatment. There was no statistically significant difference in the incidence of osteolytic changes in the acromioclavicular joint; however, patients who underwent nonoperative treatment had a significantly lower percentage of subsequent calcification of the coracoclavicular ligament.

Improvement of diagnostic precision and establishment of a new therapy for giant cell tumor

We often have difficulty distinguishing giant cell tumor, which presents with a variety of magnetic resonance findings, from other tumors. Our study results suggest that combining ultrasonography and cystography with magnetic resonance may increase diagnostic precision. Giant cell tumor of bone has a high rate of recurrence after surgery and is often difficult to resect completely. For such reasons, novel therapies are needed. The receptor activator of nuclear factor kappa B (RANK) ligand-RANK pathway has been shown to be involved in giant cell tumor proliferation and survival. Therefore, we believe an antibody against RANK ligand might be used to treat giant cell tumor of bone.

Peak height velocity as a predictive factor for curve progression in patients with late-onset idiopathic scoliosis

The aim of this study was to investigate the relationship between the magnitude of the Cobb angle at peak height velocity and last treatment method in female patients with late-onset idiopathic scoliosis. Our findings indicate that a 32-degree curvature at peak height velocity is a significant predictive factor indicating curve progression to a magnitude requiring surgical treatment.

An investigation of radiographic changes and axial symptoms after cervical laminoplasty

We have examined axial symptoms and radiographic changes after cervical laminoplasty. The results suggest the possibility of maintaining sagittal alignment by preserving the C2 semispinalis cervicis muscle. We observed no difference in the severity of axial symptoms.

The efficacy of surgical treatment for infectious spondylitis with minimally invasive spine stabilization

The aim was to evaluate clinical outcomes of minimally invasive spine stabilization with the percutaneous pedicle screw system in the treatment of infectious spondylitis. The results showed that the minimally invasive spine stabilization procedure is extremely useful for treating infectious spondylitis.

Cementless femoral reconstruction in revision total hip arthroplasty: A comparison between modular and interlocking stems

Short-term clinical results of cementless femoral reconstructions in 120 revision total hip arthroplasties were evaluated, and outcomes of 2 stem systems were compared. Use of S-ROM stems (DePuy, Johnson & Johnson, Warsaw, IN, USA) was frequently complicated by fractures, but biological fixation was consistently achieved. Good long-term results could apparently be expected if meticulous treatment of the complicated fractures was not neglected. The interlocking stem, on the other hand, could be used safely with a low incidence of complications, even in femurs with severe bone defects. However, proper biological fixation was difficult to obtain because bone apposition around the proximal stem was usually not sufficient. We speculate that this problem can be solved with the interlocking stem to obtain long-term successful outcomes.

Patient-matched instrumentation method in total knee arthroplasty: a prospective study of the accuracy of different patient-specific bone cutting guides

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

Treatment of navicular fractures of the foot

We reviewed the treatment of rare navicular fractures of the foot. A few fractures were isolated fractures, but most were accompanied by neighboring fractures or dislocations. With comminuted fractures of the navicular body, the medial column shortened owing to collapse of the articular surface or upper displacement of the fragments. In these fractures, adequate reduction and restoration of the original bone shape are generally difficult; therefore, bone collapse or arthrosis or both occasionally occur as complications after treatment. We believe that plate fixation or external fixation provides a satisfactory bone configuration with only mild postoperative pain.

Effects of microporous structure and local administration of recombinant human BMP-2 on bioresorption of β -TCP and new bone formation

Our study demonstrates that most collagen fibrils are located within micropores at an early stage following implantation of β -TCP. These findings suggest that micropores provide an environment for collagen formation, leading to deposition of apatite crystals, and indicate that micropores play important roles in β -TCP resorption and new bone formation.

Recombinant human (rh) BMP-2 induces bone formation but also stimulates bone resorption. The resorption rate of the β -TCP treated with rhBMP-2 was higher at every stage

of osteogenesis. The rate of new bone formation of β -TCP treated with rhBMP-2 was also significantly higher than without rhBMP-2 at 12 weeks. Thus, we have demonstrated that local rhBMP-2 administration accelerates both osteoclastic resorption of β -TCP and new bone formation.

A novel evaluation system to monitor bone formation and β -TCP resorption in opening high tibial osteotomy

The resorbability of β -TCP in osteotomy sites has not been well studied. The aim of this study was to establish an evaluation system to monitor bone formation and β -TCP resorption. Thirty-one patients were enrolled for evaluation. All patients underwent computed tomography (CT) at 2 weeks and at 6 years after surgery. The CT image data were divided into 4 areas, and the CT values for each area were analyzed with Osirix imaging software. The CT image analysis showed that β -TCP with 75% porosity was completely resorbed and replaced by bone. The β -TCP with 60% porosity was resorbed but approximately one-third remained even 6 years after surgery. The imaging software enabled scanning of the whole area to measure CT values. This system is useful for evaluating β -TCP resorption and bone formation in opening high tibial osteotomy.

Bone quality in chronic obstructive pulmonary disease

Both chronic obstructive pulmonary disease and diabetes are associated with increased risk of fracture. On the basis of the current definition, both bone density and quality, which encompass the structural and material properties of bone, are important factors in bone strength; diabetes reduces bone quality rather than density. Another factor playing an important role in bone strength is collagen cross-linking. Collagen cross-links can be divided into lysyl hydroxylase and lysyl oxidase-mediated enzymatic immature divalent cross-links, mature trivalent cross-links, and glycation- or oxidation-induced nonenzymatic cross-links (advanced glycation end products) such as pentosidine. On the basis of the results of our case-control study, we have provided the first evidence that both chronic obstructive pulmonary disease and diabetes impair formation of bone-collagen cross-links.

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Department of Neurosurgery

Toshiaki Abe, *Professor*
 Yuichi Murayama, *Professor*
 Hisashi Onoue, *Associate Professor*
 Tatsuhiro Joki, *Associate Professor*
 Toshihide Tanaka, *Assistant Professor*
 Toru Terao, *Assistant Professor*

Satoshi Tani, *Professor*
 Satoshi Ikeuchi, *Associate Professor*
 Yuzuru Hasegawa, *Associate Professor*
 Yasuko Kusaka, *Assistant Professor*
 Yasuharu Akasaki, *Assistant Professor*
 Toshihiro Ishibashi, *Assistant Professor*

General Summary

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

Research Activities

Cerebrovascular Disorders

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

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

Endovascular surgery

1. Development of a new endovascular operating system

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

2. Development of a new endovascular operating system

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

3. Development of bioactive coils (Matrix coil)

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

4. Development of Mebiol gel

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

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

5. Flow dynamics for intracerebral aneurysm

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

Brain tumor

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

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

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

Neurotrauma

Few institutions have engaged in research on neurotraumatology. A unique aspect of our department is research in this area, which has 3 major topics. We examined the prevalence of sports-related head injury in collaboration with the Japan Society of Clinical Sports Medicine and the Japan Society of Neurotraumatology. We also examined sports-related concussion and performed mechanical studies of head-injury through simulations.

Syringomyelia

About 60 patients with syringomyelia are surgically treated in our department each year. We have been investigating the following subjects.

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

In syringomyelia related to Chiari malformation, the relation between cerebrospinal fluid (CSF) circulation blockage and cavitation of the spinal cord has been clarified. Therefore, the improvement of the CSF circulation becomes the goal of surgical treatment. However, the mechanism of cavitation of the spinal cord is not fully understood. In patients with Chiari malformation, the cerebellar tonsils and the ventral vector (i.e., the dens) compress the spinal cord and restrict CSF circulation. We examined whether these 2 factors influence the effects of foramen magnum decompression.

2. Fluid in the syrinx

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

Spine and spinal cord group

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

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

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

Division of Pediatric Neurosurgery

The Division of Pediatric Neurosurgery, The Jikei University Hospital Women's & Children's Medical Center, was established in October 2002. In the last 10 years more than 1,500 new cases of various entities have been collected and recorded in our data bank, including, spina bifida, hydrocephalus, craniofacial anomalies, and brain tumors. Since April 2003, clinical research fellows, 12 from other domestic universities and 9 from other countries (including Germany, Italy, Austria, Jordan, and Bulgaria), have taken part in our research activities.

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

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

A member of our department has been nominated as the chairman of the National Study Group on Spina Bifida and has been promoting further nationwide and international cooperative studies on controversial issues in this field.

In the field of craniofacial anomaly research, we have extensively applied the distraction method to Japan's largest series of cases; the clinical efficacy has been summarized, and our extensive work received the honorable prize of the International Society for Pediatric Neurosurgery, Raimondi's Award in 2004, and the Kawabuchi Award in 2005.

Our clinical and research activities have been well maintained both in Tokyo (The Jikei University Hospital Women's & Children's Medical Center) and in Hannover, Germany (the International Neuroscience Institute) on the basis of firm international collaboration with world-leading pediatric neurosurgeons and related research workers.

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Department of Plastic and Reconstructive Surgery

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

Takeshi Miyawaki, *Associate Professor*
Shintaro Matsuura, *Associate Professor*
Katsuya Mori, *Assistant Professor*

General Summary

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

Research Activities

Treatment of complications after free flap reconstruction for head and neck cancer

Of the 328 patients who underwent free flap reconstruction after excision of head and neck cancers from January 2005 through January 2010, 14 patients had total flap necrosis. The necrotic flaps were 7 free jejunum flaps, 3 rectus abdominis musculocutaneous flaps, 2 anterolateral thigh flaps, and 2 fibular bone flaps. As soon as the necrosis of free jejunum flaps was recognized, salvage procedures were performed. Free flap reconstruction was performed in 6 patients, and the grafts survived in 5 patients. Considering functional and cosmetic aspects, free flap retransplantation is desirable as a salvage strategy for total flap necrosis. When free flap reconstruction is difficult, a pedicled flap is an option, if some degree of function and cosmetic effects can be maintained.

Evaluation of flap vascularization with intraoperative and postoperative infrared thermal imaging

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

Ilizarov Minifixator

The Ilizarov minifixator is a useful device in various areas of hand surgery. Its clinical

usefulness was demonstrated in the treatment of fractures (open fracture, comminuted fracture, fracture adjacent to the joint), joint contractures, malunion of fractures, and pathological fractures caused by enchondroma. It was also used with good results in bone lengthening and the temporary traction of joints. Use of the Ilizarov minifixator is an effective and noninvasive method and is highly recommended in selected cases.

Distraction osteogenesis

The use of distraction osteogenesis in reconstructive surgery continues to expand and evolve. The effects of the various rates and frequencies of distraction have been studied, and a rate of 1 to 2 mm per day has been found to be adequate for the craniofacial skeleton, whereas a rate of 0.5 to 1 mm per day is suitable in the extremities. Distraction osteogenesis is especially useful in the treatment of hemifacial microsomia, brachydactyly, and differences in limb length.

Use of flaps in reconstruction of the hand

Flap surgery is frequently needed in reconstruction of an injured hand. Suitable flaps are selected according to the sites of skin and soft-tissue loss. Also required are the diagnosis and treatment of accompanying injuries of nerves, vessels, tendons, bones, and joints. Primary treatment is often the most important for the functional outcome of the hand.

Frequently used flaps are oblique triangular flaps for tip injuries, digitolateral flaps for the palmar side of the digits, and adipofascial turnover flaps and free anterolateral thigh flaps in reconstruction of the gliding floor of the extensor tendons.

Congenital anomalies associated with isolated cleft palate

A total of 183 cases of isolated cleft palate (59 in boys and 124 in girls) from 1968 through 2003 were evaluated. The average age of the patients at the first examination was 9.9 months (range, 4 days to 11 years). Congenital anomalies other than cleft palate were observed in 57 patients (31.1%; 21 boys and 36 girls). Cleft type 43 (13 boys and 30 girls) occurred in both the hard and soft palates, and cleft type 18 (8 boys and 10 girls) occurred in the soft palate only. No congenital anomalies were found in the cases of submucous cleft palate. Tongue tie was found in 7 cases, inguinal hernia in 5 cases, congenital heart diseases in 8 cases, anomalies of the extremities in 12 cases, and mental retardation in 11 cases. Pierre Robin sequence and Apert syndrome were each found in 11 cases.

Dye laser therapy for infantile hemangioma (strawberry mark)

Early dye laser treatment for strawberry marks has been performed with good results. Patients are classified by the age of first treatment into 3 groups (younger than 1 year, 1 to 2 years old, and older than 2 years) and by appearances into 2 groups (plaque type and tumor type). The results of laser treatment in each group were investigated. In all patients surface wrinkles disappeared. In patients younger than 1 year tumor-type marks tended to flatten. Our patients showed less conspicuous scar formation than did patients not receiving dye laser treatment. Strawberry marks are common

in premature infants, 7 of whom were treated. Flattening was observed in tumor-type marks, and good results were obtained in the majority of cases.

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Department of Cardiovascular Surgery

Kazuhiro Hashimoto, *Professor*
 Yuzuru Nakamura, *Professor*
 Kei Tanaka, *Assistant Professor*
 Ryuichi Nagahori, *Assistant Professor*
 Michio Yoshitake, *Assistant Professor*

Kiyozo Morita, *Professor*
 Yoshimasa Sakamoto, *Associate Professor*
 Koji Nomura, *Assistant Professor*
 Yoshimasa Uno, *Assistant Professor*
 Hirokuni Naganuma, *Assistant Professor*

General Summary

The main investigations in our department involved clinical studies, evaluation of alterations in cardiac performance, and long-term results after any type of cardiac surgery, and animal experimental studies to address current clinical problems. Clinical investigations, including introduction of new techniques and follow-up studies, of valvular and ischemic heart diseases were a main area of our clinical research, as were studies of complex congenital anomalies. A recent topic in adult surgery is the introduction of a new field — transcatheter aortic valve replacement — and we started preparing to perform such operations. The recent increase in aortic aneurysms has continued, and a surgical strategy has been established and has achieved good results. These good results are apparently related to collaboration with the Division of Vascular Surgery. We are also performing several experimental studies with *in vivo* models. Our experimental projects include protection of the heart during cardiac arrest and pulmonary valve function. The major activities are described below.

Research Activities

Clinical investigation of myocardial protection during pediatric heart surgery

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

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

1. Effect of ischemic postconditioning and remote preconditioning: Experimental study in an *in vivo* piglet model of the reversal of myocardial stunning by ischemic postconditioning and remote preconditioning

This study tested the hypothesis that ischemia/reperfusion-induced myocardial damage can be reduced by postconditioning at reperfusion. Eighteen piglets underwent 90 minutes of ischemia followed by 60 minutes of reperfusion on cardiopulmonary bypass. In 12 of them, ischemic postconditioning strategies — 6 cycles of 10-second ischemia/reperfusion (PC-I) or 3 cycles of 30-second I/R (PC-II) — were applied before aortic unclamping, whereas the other 6 were not treated (control). Left ventricular (LV) sys-

tolic and diastolic dysfunction associated with oxidant-induced biochemical injuries were noted in the control group. In contrast, postconditioning led to significantly better LV functional recovery and less myocardial biochemical injury. On the basis of this study we concluded that ischemic postconditioning at the early phase of reperfusion produces prompt myocardial functional recovery with less biochemical injury in a piglet model of cardiopulmonary bypass. Furthermore, to develop clinically applicable strategies of conditioning after myocardial ischemia, we tested the efficacy of “remote” conditioning by means of ischemia-reperfusion of the abdominal aorta just before myocardial reperfusion.

Clinical studies of pediatric heart surgeries

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

There is still no consensus concerning the postoperative use and duration of anticoagulation therapy with warfarin for patients with Fontan circulation. We evaluated changes in coagulability and fibrinolytic function after surgery and tried to use these changes as indicators for anticoagulation therapy. Plasma levels of thrombin-antithrombin 3 complex (TAT) as an index of coagulability and levels of $\alpha 2$ -plasmin inhibitor-plasmin complex (PIC) as an index of fibrinolytic function were measured in 16 patients with extracardiac Fontan circulation (mean age at operation, 4.2 years). The levels of both TAT and PIC remained higher than normal within 6 months after surgery, even in patients receiving warfarin. The levels of both TAT and PIC began to gradually decline and had almost normalized by 12 months. Confirming these results, we have changed from warfarin to antiplatelet agents for such patients. Even after this change, plasma levels of TAT and PIC have remained normal, and no patients had evidence of thromboembolic events on echocardiography. This study suggests that patients with Fontan circulation require anticoagulation therapy with warfarin for the first postoperative year, because of activated coagulability. However, warfarin can be replaced with an antiplatelet agent for patients with normal levels of TAT and PIC and no major complications more than 12 months after surgery.

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

We have retrospectively analyzed the effect of treatment with oral pulmonary vasodilators (sildenafil and bosentan) on the hemodynamic risk profile (pulmonary arterial pressure and pulmonary vascular resistance) in 8 high-risk candidates for the Fontan procedure and in 10 control patients not treated with oral pulmonary vasodilators. In the 8 patients who received oral pulmonary vasodilators, significant reductions in pulmonary vascular resistance and pulmonary arterial pressure were noted 6 and 12 months after the bidirectional Glenn operation, whereas no changes were seen in the control group. This study suggests that treatment with oral pulmonary vasodilators (sildenafil and bosentan) improves pulmonary risk factors in candidates for the Fontan procedure.

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

The surgical outcomes and long-term results of the Ross operation were reviewed in 35

patients from 1995 through 2008. Autograft function was assessed postoperatively with periodic echocardiographic examinations for up to 14 years. There were no operative or acute deaths or late reoperation for patients with regurgitation after receiving autografts. The rate of freedom from reoperation for autograft failure was 87% over 14 years. The implanted pulmonary autograft valve showed excellent durability, especially in pediatric patients and patients with preoperative aortic stenosis.

Clinical study of adult cardiac surgery

1. Therapeutic strategy for patients older than 80 years with aortic stenosis in the transcatheter aortic valve implantation generation

Reflecting the recent aging society, nearly 10% of valve operations are performed in elderly, high-risk patients who are candidates for transcatheter aortic valve implantation. The operative risk is approximately 5%, and the surgical outcomes of aortic valve replacements have been satisfactory. There have been no patients for whom cardiopulmonary bypass was contraindicated and no serious perioperative complications. At present, surgical indications should be decided on the basis of the preoperative condition, risk scores, and other factors.

2. Choice of surgical procedures according to cause of degenerative mitral valve regurgitation: From Barlow's disease to fibroelastic deficiency

Surgical strategies should be devised on the basis of the cause of degenerative mitral valve regurgitation, from Barlow's disease, the incomplete type, and the fibroelastic deficiencies. The surgical technique of triangular resection is adapted to anterior leaflet prolapse, and the area of resection is limited as much as possible to the rough zone. Artificial chordal reconstruction is used in addition to leaflet resection if the prolapsed area is wide. In the posterior prolapse, the triangular resection is adapted, and the defect is closed with simple sutures. The diseased prolapsed area between the healthy supported chordae is removed, but the annular lesion should not be cut. The commissural small prolapse can be corrected with an edge-to-edge procedure, and the resection and suture procedure should be adapted to the larger commissural prolapse. In most cases, the flexible saddle rings are sutured for the annuloplasty. If the posterior leaflet height is supposed to be long, partial rings may be suitable for avoiding systolic anterior movement. The ring size is decided on the basis of the surface of the anterior leaflet and the distance between the anterior and the posterior fibrous triangles. Eighty percent of our cases were of the fibroelastic deficiency type, which can be repaired with quadrangular or triangular resection alone without sliding techniques. The resection and suture surgical techniques for removing the diseased prolapsed areas are beneficial for avoiding the recurrence of regurgitation, but there is still an increased tendency for reoperation in cases, such as cases of Barlow's disease, with anterior wide prolapse. We must select more suitable and effective procedures for each case.

3. Heart team: The role of the perioperative heart team, composed of the intensive care unit physicians and rehabilitation staff, in dealing with patients receiving hemodialysis who underwent coronary artery bypass graft surgery

Recently, the number of patients who are receiving hemodialysis and undergo coronary artery bypass graft surgery has increased. However, the operative and morbidity risks in

patients receiving hemodialysis are high. At our institution, interdisciplinary heart teams have contributed to excellent operative outcomes. The perioperative management of patients has been decided by the heart team after daily discussions of treatment plans. Total perioperative care in the intensive care unit has helped relieve the burden on the general wards.

4. Thoracic aneurysm surgery: Preventing cerebrovascular complications in aortic arch replacement surgery

The first choice of an aortic infusion line is an ascending aorta without calcification. If the condition of the aorta is unsatisfactory, we select an axillary artery for aortic infusion. However, because this infusion is insufficient for preventing cerebral infarction during surgery, we also cannulate the atheromatous arch branch vessels under sufficient backflow that can be achieved with selective cerebral perfusion, following initial retrograde cerebral perfusion with a flow rate of 300 ml/minute for several minutes. To prevent operative cerebral infarction and air embolism, retrograde cerebral perfusion is a beneficial, safe, and simple procedure for brain protection.

5. Management of postoperative infections: New problems and steps concerning postoperative mediastinitis

The increasing numbers of patients with diabetes or chronic kidney dysfunction has increased the number of patients at high risk for surgical site infection (SSI). Such SSIs are a heavy burden for both patients and hospitals. Our basic strategies to prevent SSI are as follows: 1) check the nasal cavity, 2) have the patient take a shower, 3) shave the patient in the operating room, 4) sterilize the body surface with benzyl chloride alcohol and iodine, 5) infuse antibiotics (cephazolin) every 3 hours during the operation and continue 3 times a day for 4 days after the operation, 6) use double-layered gloves and change every 3 hours during the operation, 7) thoroughly irrigate the surgical incision with water and use absorbable monofilament sutures and single stitching if possible, 8) control the blood glucose level with continuous infusion of insulin, 9) routine postoperative control by the infection-control team and the dental team, and 10) early postoperative rehabilitation by physical therapists. This method of postoperative management has decreased the postoperative complication of mediastinitis. Recently, a new type of postoperative SSI, which appears after discharge in patients with diabetes mellitus who are unaware of the disease or live alone, has become more frequent. For these patients, it is also important to intensify close preoperative surveillance and educate them regarding the management of the surgical wound and sanitary conditions after discharge. These efforts have led to good results.

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Department of Obstetrics and Gynecology

Aikou Okamoto, *Professor*
 Kazuhiko Ochiai, *Professor*
 Seiji Isonishi, *Professor*
 Naoki Kamiya, *Professor*
 Kuniaki Ohura, *Associate Professor*
 Kyosuke Yamada, *Associate Professor*
 Kouhei Sugimoto, *Assistant Professor*
 Nozomu Yanaihara, *Assistant Professor*

Kazunori Ochiai, *Professor*
 Hiroshi Sasaki, *Professor*
 Takekazu Onda, *Professor*
 Shigeki Niimi, *Associate Professor*
 Hirokuni Takano, *Associate Professor*
 Satoshi Takakura, *Assistant Professor*
 Hiroshi Tanabe, *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. Identification of a novel long noncoding RNA involved in the tumorigenicity of ovarian clear cell carcinoma

Ovarian clear cell carcinoma (CCC) has a poor prognosis because of its chemoresistance. Therefore, identifying novel therapeutic targets is important. In this study, we have identified ASBEL, a novel antisense-noncoding (nc) RNA of the ANA/BTG3 gene, that is required for the tumorigenicity of CCC. Knockdown of ASBEL induces apoptosis in the CCC cell line JHOC5 and reduces the tumorigenicity of CCC in xenograft experiments. When ASBEL was knocked down, protein level of ANA was increased whereas ANA messenger (m) RNA didn't change. The ANA mRNA is retained in the nucleus by forming duplexes with ASBEL. When ASBEL was knocked down, ANA mRNA translocates to the cytoplasm and ANA protein is translated. Through this mechanism, ASBEL suppresses the functions of tumor suppressor gene ANA. Thus, ASBEL is a promising target for CCC diagnosis and therapy.

2. A pilot study of CD147 protein expression in epithelial ovarian cancer using monoclonal antibody 12C3

CD147 is a membrane glycoprotein that is expressed in various cancer cells and is involved in tumor invasion and metastasis by inducing stromal fibroblastic cells to produce matrix metalloproteinases. This study was performed to evaluate the relationship between CD147 expression and various clinicopathologic variables, including histological grade and survival, in a small sample of human ovarian cancers. Paraffin-embedded surgical tissue samples from 25 patients with ovarian serous and endometrioid adenocarcinomas were stained with an anti-CD147 antibody for immunohistochemical analysis. The CD147 protein was expressed in 84.0% (21 of 25 cases) of cancerous lesions but not in normal lesions. The CD147 expression by ovarian cancer cells was negatively

correlated with overall survival but was not correlated with histological grade. These results suggest that measurement of CD147 expression will enhance our understanding of the pathophysiology of epithelial ovarian cancer.

3. Promising therapeutic target of the interleukin 6 signaling pathway in ovarian CCC
Cytokine expression in a tumor microenvironment can affect both host defense against the tumor and tumor-cell survival. We have previously reported that ovarian CCC shows a dominant helper T type 2 cytokine expression pattern driven largely by interleukin (IL)-6 expression. The unique cytokine expression pattern found in CCC may be involved in the pathogenesis of this subtype. Modulation of IL-6 expression or its related signaling pathway may be a promising treatment strategy for CCC.

4. Feasibility study of paclitaxel plus carboplatin in patients with endometrial cancer: a Japan Kanto Tumor Board study

The optimal chemotherapy regimen for patients with endometrial cancer has not been established. We assessed the feasibility of postoperative chemotherapy with paclitaxel plus carboplatin for patients with endometrial cancer. Patients with newly diagnosed endometrial cancer received paclitaxel (180 mg/m²) and carboplatin (area under the curve, 6 mg/mL/minute) every 3 weeks. Treatment was continued until disease progression or the completion of 6 cycles. Toxicities were evaluated every cycle according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. Sixty patients were registered from December 2005 through November 2006. Forty-four of 60 (73.3%) patients completed all 6 planned cycles. Grades 3 and 4 hematologic toxicities were: leukopenia (61.7%), neutropenia (95.0%), anemia (21.7%), and thrombocytopenia (5.0%). Neutropenia caused 6 patients to discontinue treatment. Grade 3 nonhematologic toxicities were: nausea (3.3%), vomiting (1.7%), neuropathy (5.0%), myalgia (6.7%), and constipation (1.7%). No grade 4 nonhematologic toxicity occurred. We believe postoperative chemotherapy with paclitaxel plus carboplatin is feasible for patients with endometrial cancer.

5. Development of a second-generation photodynamic therapy for cervical cancer to reduce photosensitivity and shorten the length of stay

Uterine cervix conization has become the standard uterus-preserving treatment for early-stage cervical cancer. However, because conization increases the risks of subsequent premature birth, low birth weight, and cesarean delivery (as reported in *The Lancet*, 2006), the 2011 guidelines of the Japan Society of Gynecologic Oncology for the treatment cervical cancer call for patients to be fully informed of these risks before undergoing conization. On the other hand, photophrin photodynamic therapy (PDT) for cervical cancer is associated with a high complete-response rate (97%) and low obstetrical risk, but PDT is not recommended as the standard treatment for cervical cancer because of the significant photosensitivity induced by photophrin and the long hospital stay, which can be 3 weeks. Therefore, to develop a second-generation PDT for cervical cancer with reduced photosensitivity and shorter hospital stays, we tested, in collaboration with Professor Kunio Awazu of the Osaka University Department of Engineering, a semiconductor laser (PD laser) connected to an existing probe for uterine cervix irradiation. First, we developed a fibre channel adapter to connect an irradiation probe for lung cancer to an existing probe for uterine cervix irradiation. We then connected the main apparatus of

the PD laser to a direct irradiation probe for lung cancer, to which we connected to the uterine cervix irradiation probe in tandem through the fibre channel adapter. We performed laser irradiation under different experimental conditions. Next year, we are planning to perform a phase I clinical trial of second-generation PDT with the photosensitizer talaporfin sodium (Laserphyrin, Meiji Seika Pharma Co. Ltd., Tokyo) to produce less photosensitivity.

6. Malignant ovarian tumor in pregnancy

The aim of this study was to investigate the effect on pregnancy of the histological findings of malignant ovarian tumors. This retrospective study involved 41 patients treated for ovarian malignancy during pregnancy from 1985 through 2010. The median age of patients was 30 years (age range, 20 to 41 years). Disease stage was stage I in 38 patients (92%) and stages II, III, and IV in 1 patient (2%) each. The ovarian malignancies were borderline malignancy in 23 cases (56%), epithelial ovarian cancer in 10 cases (24%), germ cell tumors in 7 cases (17%), and a sex cord stromal tumor in 1 case. All patients underwent primary surgery: cystectomy in 6 patients (14%), unilateral salpingo-oophorectomy in 32 patients (78%), and hysterectomy with bilateral salpingo-oophorectomy in 3 patients (7%). The outcomes of pregnancy were delivery of a live newborn in 29 cases (70%), which included 21 cases of borderline tumors (91%), 2 cases of ovarian cancer (20%), and 6 cases of nonepithelial tumor (75%); termination to perform the standard treatment for ovarian malignancy in 8 cases; and spontaneous abortion in 2 cases. In pregnant women, ovarian cancer was less frequent than in an age-matched, statistically corrected control cohort of nonpregnant women based on a Japanese annual report (10 of 33 [30%] vs. control [6%]; ovarian cancer/[ovarian cancer + borderline tumor], $P = 0.001$). Pregnant women with ovarian cancer preferred to prioritize treatment at the expense of their offspring, while those with borderline tumors or nonepithelial tumors were more likely to successfully deliver live newborns.

Perinatology

1. Multiple injections of anti-mouse $\beta 2$ -glycoprotein 1 antibody induce FcR gamma-dependent fetal growth restriction in mice

Antiphospholipid syndrome (APS) is characterized by the presence of circulating antiphospholipid antibodies (aPLs). It is also a leading cause of thromboembolic events, repeated miscarriages, and fetal loss and is a major risk factor for fetal growth restriction (FGR) and pre-eclampsia. Anti- $\beta 2$ glycoprotein I ($\alpha \beta 2$ GPI) antibody is a human aPL that is considered a specific and important marker for APS. We developed a murine model of FGR by administering multiple injections of WBCAL-1, a well-characterized mouse $\alpha \beta 2$ GPI monoclonal antibody. Administration of WBCAL-1, but not of the control antibody of the same isotype and saline, into pregnant mice decreased the size of fetuses and placentas without affecting the number of delivered pups. Also, a significant increase in urinary albumin and electron microscopic changes, such as splitting layers of basal membranes in the placental labyrinth and rearrangement of pores in glomerular endothelial cells, were observed in WBCAL-1-treated mice. However, injection of WBCAL-1 did not induce any changes in blood pressure or in typical indicators of blood thromboembolic symptoms. Furthermore, our present findings suggest that proteinuria

is a symptom associated with APS-related FGR with placental and renal tissue injuries and that FcR-gamma is a molecular target for preventing $\alpha\beta 2\text{GPI}$ antibody-mediated obstetric pathologies.

2. Cytotrophoblast alterations in placentas from disorders associated with aPLs and dys-regulated clotting factors

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

3. Pathological characteristics of placentas obtained from women with obstetrical complications caused by aPLs

We investigated the effect of aPLs on the biology of the villous trophoblast in placentas stained with antibodies against Ki-67 and cytokeratin 7 to selectively identify proliferating villous cytotrophoblasts. Images were randomly selected, and cytotrophoblasts were counted. Although cytotrophoblast proliferation was reduced in all placentas, the total number of cytotrophoblasts decreased only in placentas from aPL-positive women. Hence, we suggest that aPLs inhibit cytotrophoblast proliferation throughout pregnancy.

Reproductive endocrinology

In 2012 we researched infertility, recurrent pregnancy loss, and endoscopic surgery. We assessed infertility treatments and perinatal outcomes after endoscopic surgery for patients of reproductive age. We found that endoscopic surgery did not have severe adverse effects on treatments for infertility or perinatal outcomes in patients of reproductive age.

We investigated infertility counseling. Our “Infertility Class” was seen as providing information about ending treatment for infertility. We recognized that genetic information and emotional support in the termination of treatment were of high importance in counseling by physicians and that the way genetic information is provided by each expert, such as counselors, nurses, and medical geneticists, should be discussed.

We investigated the relationship of anti-Mullerian hormone (AMH) with the cumulative pregnancy rate and the abortion rate. AMH might be used as a marker for the cumulative pregnancy rate. The abortion rate might be lower among cases with higher levels of AMH.

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Department of Urology

Shin Egawa, *Professor*
 Koichi Kishimoto, *Professor*
 Hiroshi Kiyota, *Professor*
 Nozomu Furuta, *Associate Professor*
 Takashi Hatano, *Assistant Professor*
 Akira Furuta, *Assistant Professor*

Shoichi Onodera, *Professor*
 Isao Ikemoto, *Professor*
 Koji Asano, *Associate Professor*
 Yasuyuki Suzuki, *Associate Professor*
 Kenta Miki, *Assistant Professor*
 Takahiro Kimura, *Assistant Professor*

General Summary

We performed both basic and clinical research in the following areas: oncology, involving such sites as the kidney, bladder, prostate and testes; anatomy, physiology, and pharmacology of the bladder and urethra; imaging and radiology; infections and inflammation of the genitourinary tract, such as interstitial cystitis and prostatitis; infertility; andrology and sexual function; lithiasis; technology and instruments, such as laparoscopy; transplantation; and female urology.

Research Activities

1. Basic research: We performed several projects to elucidate the biology of urological malignancies and to develop new therapeutic tools. The results of most projects were reported at the annual meeting of the Japanese Urological Association and the American Urological Association. The projects are as follows.

- 1) TMPRSS2-ERG fusion in Japanese patients with prostate cancer.
- 2) Establishment and biological analysis of our new prostate cancer model, named JDCaP, derived from a Japanese patient
- 3) Analysis of transient receptor potential A1 involved in pelvic organ cross-sensitization in rats
- 4) Urinary continence mechanisms in rats
- 5) Common variants associated with prostate cancer susceptibility in Japanese men

2. Clinical research: Several clinical studies are on going in our institution. The results of several studies have already been reported at the annual meetings of the Japanese Urological Association and the American Urological Association.

- 1) Clinical study of high dose rate brachytherapy with external beam radiation therapy for high-risk prostate cancer
- 2) Study of deep venous thrombosis after urological surgery
- 3) Study of the incidence of latent prostate cancer
- 4) Clinical study of nomograms for predicting unilateral pathological T3 prostate cancer
- 5) Clinical study of 3-dimensional image construction of positive surgical margins in patients with prostate cancer
- 6) Study of cryoablation therapy for patients with small renal tumors
- 7) Reduced expression of stem cell marker CD44v9 in urothelial basal cells in patients with interstitial cystitis/bladder pain syndrome

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Department of Ophthalmology

Hiroshi Tsuneoka, *Professor*
 Keigo Shikishima, *Professor*
 Genichiro Takahashi, *Associate Professor*
 Kazushige Toda, *Associate Professor*
 Tadashi Nakano, *Assistant Professor*
 Tsutomu Sakai, *Assistant Professor*
 Katsuya Mitooka, *Assistant Professor*
 Koichi Kumegawa, *Assistant Professor*

Osamu Taniuchi, *Professor*
 Hisato Gunji, *Associate Professor*
 Satoshi Nakadomari, *Associate Professor*
 Masaki Yoshida, *Assistant Professor*
 Akira Watanabe, *Assistant Professor*
 Takaaki Hayashi, *Assistant Professor*
 Takuya Shiba, *Assistant Professor*
 Yoichiro Masuda, *Assistant Professor*

General Summary

The main research interest of our department is the pathophysiology of the visual processing system. The following topics are the subjects of basic and clinical studies: cataract, neuro-ophthalmology, ocular oncology and histopathology, biochemistry, functional magnetic resonance imaging (fMRI), glaucoma, electrophysiology, diabetes, vitreoretinal diseases, age-related macular degeneration, uveitis, color vision, and the cornea.

Research Activities

Cataract

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

Neuro-ophthalmology

1. We provided optimized protocols for examining the rat optic nerve using high-field and high-resolution MRI. The results would be useful for evaluating optic neuropathy in experimental animal models.
2. Ten Japanese patients with the anti-aquaporin-4 (AQP4) antibody-positive neuromyelitis optica (NMO) and 255 healthy control subjects were recruited to examine the association of genetic variation of the AQP4 gene with the susceptibility to NMO in a Japanese population. We found that the AQP4 promoter polymorphism is significantly associated with an increased risk of NMO.
3. We identified 2 Japanese families with dominant optic atrophy in whom 2 mutations of the OPA1 gene (IVS20+1 G>A and V942fsX966) were detected in affected patients. Mutations in the OPA1 gene may be a major cause of dominant optic atrophy

in Japanese patients.

4. Diffusion tensor MRI is a powerful tool to visualize the optic radiation. Probabilistic tractography was used to visualize the construction of the optic radiation, especially Meyer's loop, in great detail. Recently developed methods of phase imaging and multi-shot diffusion-weighted imaging were also used to evaluate the visual pathway in patients and healthy control subjects.
5. On the basis of recent evidence-based medicine and guidelines, we reviewed medical treatment for neuro-ophthalmic disorders, including optic neuritis, neuromyelitis optica, chronic relapsing inflammatory optic neuropathy due to autoimmune optic neuropathy, optic nerve involvement in sarcoidosis, traumatic optic neuropathy, ischemic optic neuropathy, and relapsing orbital myositis. Additionally, we began a multicenter, randomized controlled trial for nonarteritic anterior ischemic optic neuropathy and a study in our department of the efficacy of long-term, low-dosage steroid therapy for ocular myasthenia gravis.
6. We reported clinical features and evaluation using 3-dimensional true fast imaging with steady-state precession and 3-dimensional time-of-flight magnetic resonance angiography in superior oblique myokymia.
7. We reviewed the contraindications for steroid pulse therapy in traumatic optic neuropathy, the clinical features of scintillating scotoma and photophobia, and emergencies in oculomotor disorders.
8. We reported atypical cases of Leber's hereditary optic neuropathy preceded by bilateral idiopathic optic neuritis, unilateral papilledema with normal cerebrospinal fluid pressure, a limited form of neuromyelitis optica with a lesion of the trochlear nerve nucleus, and optic neuritis with hypophysitis. These reports described the etiologic relationship and were highly suggestive.

Ocular oncology and histopathology

1. Rosai-Dorfman disease is a rare disorder characterized by benign and reactive proliferation of distinctive histiocytes and manifests as chronic painless cervical lymphadenopathy, also known as sinus histiocytosis with massive lymphadenopathy. Several patients have extranodal manifestations. We reported a rare case of orbital involvement of Rosai-Dorfman disease. Pathological analysis revealed lymphophagocytosis (emperipolesis), which is characterized by histiocytes showing phagocytosed viable lymphocytes.
2. We lectured on the precise anatomy of the orbit; diagnostic approaches based on demographics, frequency, clinical features, and radiological imaging aspects; and therapeutic strategies, including detailed surgical techniques, in orbital inflammation and orbital tumors.

Glaucoma

1. The purpose of treatment in patients with glaucoma is to maintain visual function and to reduce the intraocular pressure (IOP). We have used eyedrops as a medical treatment and usually pursue an operative treatment only when glaucoma is refractory to eyedrops. On the other hand, surgery for glaucoma changes the shape of the cornea, exac-

erbrates astigmatism, and decreases visual acuity. Astigmatism can be divided into cases that can and cannot be corrected with lenses. Therefore, it is most important to examine what type of astigmatism is increasing. Recently, a device for analyzing the shape of the cornea was developed and has allowed detailed measurement of astigmatic quality. We are performing examinations with Orbscan (Bausch & Lomb Surgical, Rochester, NY, USA) and the OPD Scan corneal analyzer (Nidek Co., Ltd., Gamagori, Japan).

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

3. Numerous studies have shown that human IOP in the sitting position is high in the morning and low in the afternoon and evening. When the subject is lying flat IOP increases by as much as 2 to 6 mmHg in both healthy persons and in patients with glaucoma. Recent data incorporating the concept of the habitual body position — sitting during waking hours and supine during sleeping hours — have demonstrated that peak IOP is most likely to occur at night while the patient is supine. The progression of visual field damage in normal-tension glaucoma is associated with IOP in the supine position and the magnitude of IOP elevation accompanying postural changes. It would be beneficial if treatment options were available that could specifically decrease the supine IOP, resulting in a reduction in the magnitude of IOP fluctuation caused by postural change.

However, treatment with timolol maleate, latanoprost, or brinzolamide lowers IOP in both the sitting and supine positions but does not alter the response of IOP to postural change. The postural response is also reportedly unaffected by trabeculectomy without mitomycin C and argon laser trabeculoplasty. In patients with primary open-angle glaucoma or normal-tension glaucoma, we evaluated the postural change in IOP following trabeculectomy with mitomycin C. The IOP was measured with a pneumatometer after 5 minutes with the subject in the sitting position and after 10 minutes with the subject in the supine position. Sitting IOP and 10-minute supine IOP were 10.2 ± 3.3 mmHg and 13.7 ± 4.5 mmHg, respectively. The difference between 10-minute supine IOP and sitting IOP ($\Delta\text{IOP}_{10\text{min}}$) was 3.43 ± 1.8 mmHg ($p < 0.05$). Sitting IOP and $\Delta\text{IOP}_{10\text{min}}$ were significantly correlated ($r = 0.66$, $p < 0.0001$). The lower the sitting IOP was, the lower $\Delta\text{IOP}_{10\text{min}}$ was.

Functional neuroimaging

Patients with glaucoma were examined with voxel-based morphometry to confirm several

structural changes in the visual pathway. The chiasm was evaluated with 3-dimensional T1-weighted images, and the structure of the optic radiation was evaluated with diffusion-tensor images. Both types of image were acquired with a clinical MR scanner. Fifteen patients with glaucoma and 15 age-matched healthy volunteers were recruited. A significant signal decrease was observed with voxel-based morphometry in parts corresponding to the optic chiasm and the optic radiation in patients with glaucoma. Thus, these findings suggest that in glaucoma structural changes of the visual pathway occur in intracranial structures as well as in the eyeball.

Developmental functional abnormality

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

Visual neuropsychology

A study of visual cortex function in patients with retinal degeneration

We used fMRI to compare responses in the primary visual cortex (V1) in patients with congenital or acquired retinal degeneration. In patients with congenital retinal degeneration, we observed task-independent responses in the V1 lesion projection zone (V1-LPZ) due to reorganization that had occurred in the plastic critical period. However, in patients with acquired retinal degeneration, the V1-LPZ response depended on the task. This finding suggest that in the stable cortex, the cortical circuit is not reorganized.

Low vision

On the basis of results of our questionnaire survey we developed a software program, "First Step," for supporting persons with visual disabilities. This study was supported by a grant from the Ministry of Health, Labour and Welfare (The comprehensive research for disabilities [sensory disability], H22-Sensory-general-005). The theme was the development of a comprehensive rehabilitation system program for vision. We also proposed "intermediate outreach support" as a model of how to support persons with visual disabilities in the next generation. The data showed that the activities of daily living and quality of life depend on the visual field, rather than on visual acuity.

Vitreoretinal diseases

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

capability of conventional vitrectomy.

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

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

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

Electrophysiology

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

Diabetic Retinopathy section

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

Uveitis

1. We reviewed findings of spectral domain OCT, fluorescein angiography, and indocyanine green angiography in patients with multifocal posterior pigment epitheliopathy treated with low-fluence photodynamic therapy (PDT). Early application of low-fluence

PDT for multifocal posterior pigment epitheliopathy led to restoration of photoreceptor integrity with improvement of choroidal thickness and circulation.

2. We reported the findings of angiography and OCT evaluation of steroid-associated central serous chorioretinopathy in a patient with Vogt-Koyanagi-Harada disease. Accurate understanding of angiography findings is important for establishing diagnoses, although the noninvasive OCT provides helpful information.

3. We reported a case of refractory uveitis with bilateral optic disc swelling and retinal vasculitis that was suspected to be associated with idiopathic retinal vasculitis, aneurysms, and neuroretinitis.

4. We reported the outcomes of combined phacoemulsification and pars plana vitrectomy for restoring visual acuity in patients with cataract and posterior segment involvement due to ocular tuberculosis. Results indicate that combined phacoemulsification and pars plana vitrectomy can be used to remove cataracts and pathologic vitreous in the eyes of such patients. Although the exact role of vitrectomy remains to be determined, the combined surgery successfully restored useful vision in all cases.

Macular degeneration

1. Single-session PDT combined with intravitreal bevacizumab and subtenon triamcinolone acetonide for polypoidal choroidal vasculopathy

We evaluated the efficacy of triple therapy consisting of single-session PDT, intravitreal bevacizumab, and subtenon triamcinolone acetonide as the initial therapy for polypoidal choroidal vasculopathy. We found that this triple therapy improves vision and reduces central macular thickness in polypoidal choroidal vasculopathy.

2. We described findings in spectral-domain OCT for 4 patients with acute foveal photoreceptor damage. These 4 cases of acute foveal photoreceptor damage may represent a novel clinical entity. As more cases are recognized, the characteristic features of the disease spectrum and etiology may become clearer.

Biochemistry

1. The peroxisome proliferator-activated receptor- α agonist fenofibrate has been shown to have anti-inflammatory activity and to suppress the development of experimental autoimmune encephalomyelitis. We investigated the effects of fenofibrate in experimental autoimmune uveoretinitis (EAU). The results suggest that fenofibrate modulates the development of EAU and suppress intraocular inflammation by decreasing the production of inflammatory cytokines.

2. Inhibition of extracellular signal regulated kinase (ERK) mitogen-activated protein kinase suppresses interleukin (IL)-17 production driven by IL-23- and IL-1 and attenuates autoimmune disease. We investigated the effects of the ERK inhibitors PD98059 and U0126 on EAU. The ERK inhibitors exhibited significant anti-inflammatory and immunosuppressive effects in EAU. Such ERK inhibitors are promising treatments for autoimmune uveitis.

Color vision defects and genetic analysis of retinal diseases

1. We investigated differences in color discrimination between the fellow eye and the

affected eye successfully treated for unilateral age-related macular degeneration in a 69-year-old man with protanopia (a type of dichromacy in congenital color vision defects).

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

3. We investigated the involvement of various genetic factors in Japanese patients with age-related macular degeneration, a common cause of blindness in industrialized countries. More than 500,568 single-nucleotide polymorphisms of the whole genome were genotyped with Affymetrix Human Mapping Arrays and the TaqMan assay (Affymetrix Inc., Santa Clara, CA, USA). We are now analyzing candidate single-nucleotide polymorphisms involved in Japanese patients with age-related macular degeneration.

Cornea

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

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

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Department of Otorhinolaryngology

Hiroshi Moriyama, *Professor*
 Atsushi Hatano, *Associate Professor*
 Nobuyoshi Otori, *Associate Professor*
 Makoto Iida, *Assistant Professor*
 Yuichiro Yaguchi, *Assistant Professor*
 Tetsushi Okushi, *Assistant Professor*

Takakuni Kato, *Professor*
 Hiromi Kojima, *Associate Professor*
 Shintaro Chiba, *Assistant Professor*
 Yoshinori Matsuwaki, *Assistant Professor*
 Daiya Asaka, *Assistant Professor*

General Summary

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

Research Activities

Research issues in otology

Our research projects span experiments on the fundamental aspects of middle ear mucosa regeneration and its clinical application, research on gene therapy targeting epithelium with residual cholesteatoma, and the development of a navigation system utilizing virtual-reality technology to increase the safety of surgery. In addition, cholesteatoma surgeries performed at our hospital are recorded in our database, which is used to analyze the condition of patients, to select operative methods, and to review postoperative outcomes. In regard to research on hearing loss, we are studying the physiology of the inner ear in metabolic disorders using experimental animal models and collaborating with Shinshu University in the genetic analysis of deaf patients.

We perform approximately 200 middle ear surgeries annually at our hospital. Cochlear implantations performed every year have also yielded favorable results. We perform skull-base surgery, including that for cholesteatoma in the petrous part of the temporal bone, in conjunction with the Department of Neurosurgery and have found that hearing and facial nerve function can be preserved in many cases. We also perform acoustic tumor surgery via the posterior cranial fossa approach, middle cranial fossa approach, or translabyrinthine approach, depending on the case.

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

In the field of neuro-otology, we have introduced vestibular evoked myogenic potential (VEMP) testing to evaluate saccular function in patients with such conditions as vestibulo-

lar neuritis, Meniere's disease, and dizziness of unknown cause to facilitate diagnosis and treatment. Moreover, we are examining the prevalence of abnormal saccules in various disorders as measured with VEMP testing, the ictal and nonictal phases of Meniere's disease, and the incidence of VEMP abnormalities according to disease stage. We also perform furosemide-loading VEMP as a test for patients suspected to have delayed endolymphatic hydrops. In addition, we are advancing research on the localization of the vestibular cortex and the projection from the vestibular system to the cerebral cortex by analyzing cerebral blood flow with single photon emission computed tomography in conjunction with the Department of Neurology.

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

Research in rhinology

We are involved in the analysis of data on factors related to the intractability of rhinosinusitis obtained from patients undergoing endoscopic sinus surgery (ESS) and from prospective studies of the postoperative course. We perform special care for skull-base diseases, such as pituitary tumors and cerebrospinal fluid leakage, in close collaboration with the Department of Neurosurgery. We report case studies and investigate the postoperative course of skull-base diseases. In an attempt to expand the indications for ESS from paranasal sinus tumors to skull-base surgery, including that for cerebrospinal fluid leakage, skull-base tumors, and pituitary gland tumors, and to improve the safety of ESS, we have performed high-tech navigation surgery in which 3-dimensional endoscopic images and stereonavigation images are superimposed. Furthermore, intraoperative computed tomography scan update for image-guided systems to adapt to anatomical changes during surgery is being developed. We have identified problems and possible areas of improvement relevant to this operative method and are altering the device to improve its accuracy and performance.

We have planned clinical studies and developed treatment methods for patients with a variety of olfactory disorders. We began rehabilitation for olfactory disorders for the first time in Japan. Since last year we have offered anatomy training using fresh-frozen cadavers at the Skills Laboratory, for training in both skull-base surgery and ESS. We must improve both medical techniques and our anatomical knowledge. In addition, we have started creating new methods of Internet access using telemedicine and a distance-training system. To elucidate the pathogenesis of eosinophilic chronic rhinosinusitis and allergic fungal rhinosinusitis, we investigate how environmental fungi and bacteria induce activation and degranulation of human eosinophils and the airway epithelium.

Research issues on head and neck tumors

For common advanced cancers we perform radical surgery (e.g., total pharyngolaryngectomy combined with reconstruction by means of free intestinal flap transfer for hypopharyngeal cancer and total laryngectomy for laryngeal cancer); however, we perform larynx-preserving surgery (partial hypopharyngectomy combined with reconstruction by

means of free-flap transfer and partial laryngectomy) to preserve function, especially vocal function, to the greatest extent possible. We have obtained favorable outcomes in terms of both laryngeal preservation and survival. For conservative therapy and postoperative treatment for advanced cancers, we perform radiotherapy, alone or with concurrent chemotherapy with cisplatin and fluorouracil, and have obtained favorable results. We use narrow-band imaging endoscopy for diagnosis in routine practice and make good use of this technology for the diagnosis and treatment of early-stage superficial mesopharyngeal and hypopharyngeal cancers.

In regard to research on cancer, we are performing basic studies and applying their findings to future studies and to clinical practice; such fundamental studies include extraction of DNA from specimens obtained during surgery and evaluation of epidermal growth factor receptor expression, a target for molecularly targeted agents. In the future, we hope to perform clinical research on the expression of human papilloma virus, which has been implicated in the development of mesopharyngeal cancer and oral cancer, and to investigate treatments, such as vaccine therapy, for various cancers.

Research on vocal and swallowing functions

1. Phonosurgery: We are performing outpatient day surgery using a flexible fiberoptic laryngoscope and performing laryngomicrosurgery with the microflap method and the patient under general anesthesia for vocal fold polyps, vocal cord nodules, and vocal cord cysts. To determine the optimal surgical indications and operative methods, we compare potential operative methods by means of fiberoptic laryngoscopy, stroboscopy, acoustic analysis, aerodynamic testing, and assessment using the Voice Handicap Index before and after surgery.

For many years we have performed injection of atelocollagen into the vocal folds as outpatient day surgery for unilateral recurrent-nerve paralysis; however, we are also performing laryngeal framework surgery for patients who are considered poor candidates for atelocollagen injection.

2. Diagnosis and treatment of spasmodic dysphonia: Since December 2004 we have performed botulinum toxin treatment as a first-line therapy for spasmodic dysphonia with the approval of the ethics committee of the university. The prevalence of this disorder has been increasing; therefore, evaluating methods for diagnosis and treatment is of clinical importance. An important future task in this context is developing surgical treatment methods for patients who do not respond to botulinum toxin treatment.

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

Research on sleep apnea syndrome

To verify whether allergic rhinitis is involved in sleep disorders, research for patients with pollinosis has been performed since last year at the Ota Sleep Science Center.

Continuous positive airway pressure treatment is the first choice for patients with obstruc-

tive sleep apnea syndrome of greater than moderate severity. On the other hand, the effectiveness and safety of surgical treatment are still unknown. Therefore, we investigate the roles of surgery, such as uvulopalatopharyngoplasty. We will be able to present the adaptation of surgical treatment for sleep disorders. Long-distance sleep examinations have been performed since 2009 at the Ota Sleep Science Center.

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Department of Anesthesiology

Shoichi Uezono, *Professor*
 Naohito Shimoyama, *Professor*
 Masanori Takinami, *Associate Professor*
 Chieko Fujiwara, *Associate Professor*
 Yasushi Mio, *Associate Professor*
 Masamitsu Sanui, *Associate Professor*
 Yoshie Taniguchi, *Assistant Professor*
 Yoichi Kase, *Assistant Professor*
 Yukino Kubota, *Assistant Professor*

Sachiko Omi, *Professor*
 Shuya Kiyama, *Associate Professor*
 Masaki Kitahara, *Associate Professor*
 Ichiro Kondo, *Associate Professor*
 Shigehiko Uchino, *Associate Professor*
 Takahiro Matsumoto, *Assistant Professor*
 Kazuhiro Shoji, *Assistant Professor*
 Gumi Hidano, *Assistant Professor*
 Hiroshi Sunaga, *Assistant Professor*

General Summary

The functions of the Department of Anesthesiology are to provide quality patient care, to teach, and to perform research in perioperative medicine, intensive care medicine, and comprehensive pain management. In 2012 we made further advances and great achievements with the support of our faculty, institutional administration, and the Dean of The Jikei University. Below we highlight some of our research achievements in 2012.

Research Activities

Research continues as a growing and important component of the department's activities. The department is committed to enhancing academic productivity and resources by dedicating time to research and granting clinical access to research cases.

The investigators have been successful each year in obtaining peer-reviewed research grants, such as Grants-in-Aid for Scientific Research (*kakenhi*) and contract grants. The department continues to build on the strengths of several outstanding programs: cardiovascular anesthesia, thoracic anesthesia, pediatric anesthesia, regional anesthesia, neuro-anesthesia, obstetric anesthesia, intensive care medicine, and comprehensive pain management. Faculty recruitment is targeted at individuals with demonstrated academic and research activities as well as excellent clinical management and teaching skills. In 2012, the department was able to invite Naohito Shimoyama, M.D., from Tokyo Medical College as a full professor. Dr. Shimoyama is a nationally recognized expert in palliative medicine. He is expected to develop comprehensive palliative care for cancer patients, a first for The Jikei University, in close collaboration with our existing outpatient pain clinic and acute pain service. Our faculty and residents were both well represented at the Japanese Society of Anesthesiologists' annual meeting in Sapporo and at the American Society of Anesthesiologists' annual meeting in Washington, D.C. In addition, members of the department continue to be invited as visiting professors or guest speakers at national and international meetings. Listed below are the ongoing research projects in which the principal investigators are faculty members of the Department of Anesthesiology.

Doctor Hidano's research focus has been on searching for preoperative factors to predict intraoperative and postoperative deep vein thrombosis in surgical patients. This study will help us identify patients at high risk for postoperative pulmonary emboli, which can

sometimes be fatal. Doctor Taniguchi has been interested in temperature regulation during surgery and its effects on postoperative outcomes, such as shivering. Doctor Uchino continues to be active in clinical research in the intensive care unit and has been extremely productive in the field of acute kidney injury. Our pain clinic physicians continue to play a pivotal role in establishing practice guidelines for patients with neuropathic pain. Their particular interest has been postmastectomy pain.

Basic science investigations included studies of the effects of neuromuscular blocking agents on the central nervous system (Dr. Uezono), studies of the effects of sustained release of intrathecal morphine (Dr. Kondo), mechanisms of anesthetic postconditioning in renal mitochondria (Dr. Mio), *in-vivo* nanoimaging techniques in cardiac disease (Dr. Terui), and the mechanisms of the effects of valproic acid on the treatment of chronic pain (Dr. Hobo). The appended bibliography of the department shows a wide range of investigative and scholarly activities over the past year.

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Department of Rehabilitation Medicine

Masahiro Abo, *Professor*
 Kazushige Kobayashi, *Associate Professor*
 Kun Suk Chung, *Assistant Professor*
 Itaru Takehara, *Assistant Professor*
 Tadashi Suzuki, *Assistant Professor*
 Keiji Hashimoto, *Assistant Professor*

Shu Watanabe, *Professor*
 Wataru Kakuda, *Associate Professor*
 Hideki Sugawara, *Assistant Professor*
 Masanori Funakoshi, *Assistant Professor*
 Nobuyuki Sasaki, *Assistant Professor*

General Summary

The main research topics of our department are as follows: 1) repetitive transcranial magnetic stimulation (rTMS) for stroke, 2) driving after stroke, 3) development of scales for children, 4) imaging in mild traumatic brain injury (TBI), and 5) cognitive rehabilitation.

Research Activities

rTMS for stroke

1. Effectiveness of low-frequency rTMS and intensive speech therapy in patients with aphasia after stroke: A pilot study based on evaluation with functional magnetic resonance imaging in relation to the type of aphasia

Patients with left-hemispheric stroke and aphasia were studied. Each patient received 10 treatment sessions consisting of low-frequency (LF) rTMS and intensive speech therapy. The area for stimulation was selected with functional magnetic resonance imaging. The LF-rTMS was applied to the inferior frontal gyrus for patients with nonfluent aphasiae and to the superior temporal gyrus for patients with fluent aphasiae. After treatment, patients with nonfluent aphasiae showed significant improvements in auditory comprehension, reading comprehension, and repetition. Patients with fluent aphasia showed a significant improvement in spontaneous speech only.

2. Effect of LF-rTMS on motor neuron excitability after stroke

Patients with spastic upper-limb hemiparesis after stroke were studied. The LF-rTMS was applied to the motor cortex of the nonlesional hemisphere. The amplitude ratio of mean F-wave to M-response (F-mean/M ratio) was measured before and after LF-rTMS and was analyzed for both limbs. The application of LF-rTMS significantly decreased the F-mean/M ratio in the affected upper limb but not in the unaffected limb.

3. Change in regional cerebral blood flow after LF-rTMS combined with intensive occupational therapy for upper-limb hemiplegia after stroke: A study with single photon emission computed tomography

Patients with upper-limb hemiplegia after stroke were studied with single photon emission computed tomography before and 4 weeks after treatment with LF-rTMS and intensive occupational therapy. Before and after treatment, the function of the hemiplegic upper-limb function showed significant improvement. The specific areas with significantly increased regional cerebral blood flow (rCBF) were the insula (BA 13), precentral gyrus (BA 44), and cerebellum in the affected hemisphere, and the lingual gyrus and cer-

ebellum in the nonaffected hemisphere. On the other hand, rCBF was significantly decreased in the middle frontal gyrus (BA 6), precentral gyrus (BA 4), and postcentral gyrus (BA 3) in the nonaffected hemisphere. These results suggest that the combination of LF-rTMS and intensive occupational therapy has an effect on rCBF and has contributed to the improvement of upper-limb hemiplegia after stroke.

Driving after stroke

1. Resumption of driving after stroke

In a questionnaire survey of 525 patients discharged after stroke, 118 patients responded that they drove before having a stroke, and 42 (35.6%) had resumed driving. At the time of discharge, nearly 70% wanted to start driving again. Supporting the resumption of driving after stroke is an important mission for rehabilitation specialists.

Development of scales for children

1. Evaluation of the Ability for Basic Movement Scale for Children Type T in disabled children

Children with disabilities who were able to walk participated in this study. Subjects were administered the Ability for Basic Movement Scale for Children Type T (ABMS-CT) by 2 physicians, and the results of the Functional Independence Measure for Children (WeeFIM) were recorded. Spearman's rank correlation coefficient analysis showed that both the individual scores for each item and the total scores of the ABMS-CT correlated significantly with the total scores of the WeeFIM. The items of the ABMS-CT had appropriate internal consistency and reliability.

Imaging in mild TBI

1. A new method for evaluating of mild TBI with neuropsychological impairment using statistical imaging analysis for Tc-ethyl cysteinate dimer single-photon emission computed tomography

Using 2 analytic software packages, the easy Z-score imaging system and voxel-based stereotactic extraction estimation, we identified specific lesions with low regional uptake of Tc-99m ethyl cysteinate dimer possibly associated with neuropsychological impairment (NPI) after mild TBI. This trend was most marked in the left anterior cingulate gyrus in patients with mild TBI and NPI and in patients with diffuse axonal injury. The optimal "extent" cutoff value, as a criterion for single-photon emission computed tomography abnormality, might help the diagnosis of NPI after mild TBI.

Cognitive rehabilitation

1. Evidence based cognitive rehabilitation

To establish best practice recommendations, it is necessary to assess the evidence regarding cognitive rehabilitation. This study sought to integrate a growing number of treatment guidelines recommending the delivery of evidence-based cognitive rehabilitation. 1) The guidelines suggest several interventions that may be used to treat patients with aphasia. 2) There is strong evidence that compensatory strategy training is effective in improving memory outcomes after brain injury and, therefore, improving quality

of life. 3) Direct attention training may be recommended for patients who unable to concentrate, and time pressure management significantly reduces task errors. 4) Survivors of TBI with deficits of executive functioning should receive metacognitive treatment or goal management training. 5) Patients with apraxia should be trained in compensatory strategies, e.g., verbalization and following a written/pictorial action sequence. 6) Longitudinal studies suggest that planned behavioral modification programs are effective for preventing these undesired behaviors from becoming established. 7) For patients with brain injury who want to return to work, supported employment is effective.

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Department of Emergency Medicine

Takeki Ogawa, *Professor*
 Joji Otsuki, *Associate Professor*
 Kei Ohtani, *Assistant Professor*
 Kenji Okuno, *Assistant Professor*

Tsutomu Koyama, *Professor*
 Satoshi Takeda, *Associate Professor*
 Taro Nameki, *Assistant Professor*

General Summary

1. Education system for junior residents in emergency medicine
2. Establishing a database of severe traumatic brain injury in Japan
3. The etiology of syncope
4. Research on laboratory assessment of myocardial infarction in the emergency room
5. Managing a course on immediate cardiac life support
6. Managing the Japan Advanced Trauma Evaluation and Care course
7. Providing logistical support to the Japan Boxing Commission

Research Activities

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

Publications

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Department of Endoscopy

Hisao Tajiri, *Professor*
Hiroshi Arakawa, *Assistant Professor*
Hiroo Imazu, *Assistant Professor*
Keiichi Ikeda, *Assistant Professor*
Kazuki Sumiyama, *Assistant Professor*

Tomohiro Kato, *Associate Professor*
Koji Matsuda, *Assistant Professor*
Shoichi Saito, *Assistant Professor*
Kenichi Goda, *Assistant Professor*

General Summary

Our main area of research is performing clinical studies of endoscopy in the diagnosis and treatment of gastrointestinal, hepatobiliary and pancreatic disease. In addition, we perform basic research to develop novel instrumentation, methods of image processing and analysis, and optical apparatuses, such as autofluorescence imaging (AFI), narrow-band imaging (NBI), endocytoscopy, confocal laser endomicroscopy, endocytoscopy, and therapeutic endoscopy with a high degree of procedural freedom. Our published research outcomes and recent reports are summarized below.

Research Activities

Pharyngeal, esophageal and gastric malignancies

1. Endoscopic diagnosis in esophagogastric neoplasia

Early detection and accurate diagnosis of premalignant and malignant lesions in the pharynx, esophagus, and stomach are essential to allow the most appropriate therapeutic strategy to be selected for each patient. Our research utilizes the following novel optical technologies, along with conventional white light endoscopy, in clinical cases. We have designed a series of prospective clinical studies to evaluate and validate these novel imaging technologies and their potential benefits. We introduced transnasal ultrathin endoscopy, which is expected to improve patient compliance. This type of endoscope is particularly useful for screening patients from the non-referral hospital population, as it will reduce discomfort during endoscopic examination.

1) Magnifying endoscopic observation using an NBI system

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

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

2) Endocytoscopy

Endocytoscopy is a novel optical imaging technique that allows the gastrointestinal mucosa to be visualized *in vivo* and in real time at the cellular level using a staining solution. We joined a multicenter study using endocytoscopy for diagnosing superficial esophageal squamous cell carcinoma. Moreover, we performed a single-center study to determine the optimal staining regimen for *in-vivo* endocytoscopy of normal mucosa and superficial neoplasms of the duodenum. We are now studying the characteristic endocytoscopy findings of superficial duodenal neoplasms, i.e., adenoma and mucosal adenocarcinoma.

3) AFI

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

4) Ultrathin endoscopy (transnasal endoscopy)

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

5) Endoscopic ultrasound-guided fine needle aspiration biopsy

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

2. Endoscopic treatment of esophageal and gastric malignancies

With recent advances in endoscopic diagnostic techniques and instrumentation, indications have expanded for endoscopic therapy in early gastric and esophageal carcinomas. Research on the following endoscopic therapeutic modalities is now under way to

standardize the use of these techniques for treating tumors of the upper gastrointestinal tract.

1) New indications for endoscopic treatment and endoscopic submucosal dissection

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

2) Therapeutic interventions employing innovative endoscopy systems

The multibending scope (M-scope) is a new type of endoscope that provides greater access to sites that are usually difficult to access. We have previously reported on the use of the M-scope in the treatment of tumors of the lesser curvature, greater curvature, and posterior wall of the gastric body, and the cardiac region, which are not accessible with conventional endoscopes. Studies using an M-scope with magnifying capability are now under way to develop more accurate and safer procedures. Furthermore, clinical studies using a newly developed therapeutic endoscope (R-scope), which a special mechanism allowing the forceps to move laterally and vertically, in addition to the multibending function, are proceeding to advance the potential of endoscopic therapy. We have also performed several studies using natural orifice transluminal endoscopic surgery (NOTES), including full-thickness resection, because current endoscopic treatments are directed only at mucosal diseases.

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

Many studies have demonstrated an association between *H. pylori* infection and the development of gastric cancer. However, there are still many unknown factors affecting this association. Because our department routinely performs endoscopic treatment for gastric cancer, clarification of these factors is important. Experiments concerning this association, particularly on DNA methylation due to *H. pylori* infection, have been carried out in collaboration with the Department of Gastroenterology, Toshiba General Hospital. We have also been exploring the roles of inducible nitric oxide synthase (iNOS) in the pathogenesis of *H. pylori*-associated diseases and have demonstrated that eradication of *H. pylori* plays an important role in the process of repairing disease-associated DNA

methylation and in the alteration of methylation patterns of genes in the mucosa in the 5 years following *H. pylori* eradication. Interim results have been reported at several conferences and been published in Japan as well as internationally. In addition, we have reported that diverse topographical patterns of *H. pylori*-induced iNOS expression and iNOS gene polymorphism may contribute to the development of gastric cancer caused by *H. pylori* infection.

4. Diagnosis of oropharyngeal and hypopharyngeal malignancies

Endoscopic screening with iodine staining, or Lugol chromoendoscopy, has enabled esophageal cancer to be detected at an early stage and, thus, has improved prognoses. However, this technique is difficult to perform in such locations as the oropharynx or hypopharynx. Metachronous or synchronous cancer of the oropharynx or hypopharynx has become the main factor adversely affecting the prognosis and quality of life of patients with esophageal cancer. Because detecting cancer at an early stage is important, we have found that magnifying endoscopy in combination with the NBI system has allowed hard-to-find cancers to be detected during the early stages without the need for Lugol chromoendoscopy. A multicenter randomized controlled study on the clinical value of this new combination endoscopy was performed. In addition, we performed a single-center study to evaluate endoscopic characteristics of superficial carcinoma in the pharyngeal region. These results have reported at medical congresses and in English-language medical journals.

Functional disorders of the upper gastrointestinal tract

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

Diagnosis and treatment of esophagogastric varices

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

Enteroscopy and colonoscopy

1. Diagnostic techniques

Capsule endoscopy is a breakthrough modality that can be used to detect lesions in parts of the small intestine unreachable with an ordinary endoscope system. Internationally, capsule endoscopy has been performed in more than 1 million cases before May 2011 and is highly recommended as a first-line examination to detect disease of the small intestine. However, because capsule endoscopy is purely diagnostic, we have introduced single-balloon enteroscopy, which allows biopsy and hemostasis to be performed for hemorrhagic lesions of the small intestine.

The numbers of cases of colonic cancer have increased markedly in Japan. In Europe and the United States, several studies have described the use of capsule endoscopy for examining the large intestine. In Japan, we are collaborating with 6 other hospitals to perform studies with capsule endoscopy to screen patients for colonic neoplasms.

Accurate preoperative evaluation of the degree of tumor invasion into deep layers is essential for appropriate decision-making and determining the optimal therapeutic strategy for patients with colonic lesions. Hence, to maximize our diagnostic accuracy, we utilize a magnifying endoscope with NBI and crystal violet staining or AFI technology or both along with conventional white-light observation.

2. Research in endoscopic interventions

Surgical resection has been the treatment of first choice for large, flat, elevated tumors of the colon. Recently, endoscopic en bloc resection performed with ESD (a standard treatment for gastric lesions) has been used for such colonic lesions. However, endoscopic resection of large intestinal lesions is technically difficult because of the wide lumen and the higher rate of complications, such as perforation and bleeding. Our present efforts are focused on establishing safe and reliable methods to remove large colonic lesions endoscopically and to start preliminary use of ESD. Additionally, an infrared endoscopy system has been used to evaluate the risk of bleeding from vessels located at the base of the ulcer created with ESD.

3. Capsule endoscopy and enteroscopy

Capsule endoscopy is a minimally invasive endoscopic modality that can be used to detect lesions in the small intestine which have been unreachable with traditional push-type enteroscopy. Recently, particularly in the Western countries, capsule endoscopy has been recommended as the first-line endoscopic examination for evaluating and managing obscure gastrointestinal bleeding. We have performed capsule endoscopy for 289 patients since the Japanese health insurance system began covering this procedure in April 2007. Our study found that capsule endoscopy should be performed as soon as possible following a patient visiting hospital with a complaint of melena. We are aiming to further improve the diagnostic accuracy of capsule endoscopy for evaluating obscure gastrointestinal bleeding by re-evaluating the traditional bowel preparation regimen.

4. Basic research

Hyperplastic polyp is a nonneoplastic tumor of the colon. Therefore, endoscopic treatment is not indicated on a histologic basis. However, according to recent reports in Western countries, sessile serrated lesions can develop into advanced, invasive submucosal cancers that invade deeper layers. We are now examining biological markers of

malignancy in sessile serrated lesions by means of immunohistochemical staining to evaluate whether such these lesions have malignant potential.

Pancreatobiliary endoscopy

1. Diagnosis of biliary and pancreatic diseases

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

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

2. Treatment using endoscopic techniques in pancreatobiliary diseases

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

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

Palliative care

More and more interest is being shown in palliative care. Various techniques have been developed to provide the best quality of life for critically ill or terminally ill patients. Endoscopic procedures may play an important role in palliative care, especially in supporting food intake. In our department, percutaneous endoscopic gastrostomy is performed for patients who are unable to maintain sufficient oral intake. Although percutaneous endoscopic enterostomy is conventionally not indicated for patients who have undergone gastric surgery, since 1994 we have extended the use of this procedure to include such patients and have investigated the technique's clinical usefulness in this situation. Kits for percutaneous endoscopic gastrostomy developed by us have reduced the frequency of complications associated with percutaneous endoscopic enterostomy placement. To alleviate stenosis attributable to digestive tract and bile duct tumors, we have performed endoscopic ballooning/bougienage and subsequent metallic stenting, with good therapeutic results. To reduce the pain associated with chronic pancreatitis and inoperable pancreatic cancer, we have performed transgastric celiac plexus blocks using EUS. These endoscopic procedures may greatly contribute to improving the quality of life of patients who are not candidates for radical surgery. The cost-effectiveness of these interventions is another benefit.

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Department of Infection Control

Seiji Hori, *Professor*
Hiroshi Takeda, *Assistant Professor*
Tetsuya Horino, *Assistant Professor*

Masaki Yoshida, *Assistant Professor*
Yasushi Nakazawa, *Assistant Professor*

General Summary

We demonstrated the morbidity of asymptomatic sexually transmitted infections in patients with human immunodeficiency virus (HIV) infection and of metastatic infections during *Staphylococcus aureus* bacteremia. These results suggest the necessity of an active approach for identifying latent infectious diseases. In addition, we showed the validity of new antibiotic treatments for extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and of preventive medicine for malaria. Thus, our investigations suggest new strategies to diagnose, treat, and prevent infectious diseases.

Research Activities

Asymptomatic sexually transmitted infections in HIV-infected patients

We performed a questionnaire survey about sexual behavior and a survey about urethra-pharyngeal *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in outpatients infected with HIV but without symptoms of urethritis or pharyngitis. Specimens of pharyngeal gargle (10 ml of saline) and urine were examined for *C. trachomatis* and *N. gonorrhoeae* with the strand displacement amplification method. We measured antibodies (immunoglobulins A and G) against *C. trachomatis*. We performed a survey of sexual behavior in 77 patients (mean age, 40.1 years). Although *C. trachomatis* was detected in the urine of only 1 patient, antibodies against *C. trachomatis* were detected in 46 of the 77 patients (59.7%).

Analysis of atovaquone-proguanil compared with mefloquine in the chemoprophylaxis of malaria in nonimmune Japanese travelers

Malaria is a severe, extremely common infectious disease in tropical and subtropical areas. For high-risk travelers to endemic regions, malaria chemoprophylaxis is recommended. Internationally, atovaquone-proguanil, mefloquine, or doxycycline is prescribed for malaria chemoprophylaxis. However, in 2012 atovaquone-proguanil and doxycycline were not approved for use in Japan. Therefore, the data on atovaquone-proguanil for malaria chemoprophylaxis in Japanese travelers are not clear. We analyzed data from a questionnaire survey to assess the safety and tolerability of atovaquone-proguanil and compare them with those of mefloquine for nonimmune Japanese travelers. Atovaquone-proguanil was given to 278 travelers and mefloquine to 38 travelers. The mean duration of each prophylaxis was 20.0 ± 9.6 days for atovaquone-proguanil and mefloquine for 59.0 ± 15.9 days. Nine travelers discontinued treatment (5 with atovaquone-proguanil and 4 with mefloquine), and the rate of discontinuation was significantly less with atovaquone-proguanil. The frequency of adverse events was sig-

nificantly less with atovaquone-proguanil (18.8%, 52 cases) than with mefloquine group (36.8%, 14 cases). In particular, the frequency of psychoneurotic adverse events was significantly less with atovaquone-proguanil. These results suggest that atovaquone-proguanil is better tolerated and produces fewer adverse events than mefloquine in non-immune Japanese travelers.

Drug susceptibilities of ESBL-producing Escherichia coli strains isolated from urine

We investigated the drug susceptibilities of ESBL-producing *E. coli* isolated from urine and performed genotyping of ESBLs. The genotypes of the ESBLs were the CTX-M-9 group in 75.6%, the CTX-M-1 group in 14.6%, and the CTX-M-2 group in 9.8%. All strains were sensitive to meropenem, doripenem, imipenem, flomoxef, latamoxef, sitafloxacin, cefmetazole, tazobactam/piperacillin, and amikacin. Ninety percent of the strains were sensitive to faropenem. However, 73.2% of the strains were resistant to levofloxacin. There was no relationship between drug susceptibility and the genotype of ESBLs. Antimicrobial agents should be carefully selected for the treatment of urinary tract infections caused by ESBL-producing *E. coli*.

Predictive factors for metastatic infection in patients with bacteremia caused by Staphylococcus aureus

Metastatic infections, such as infective endocarditis and psoas abscess, are severe complications of *S. aureus* bacteremia, because failure to identify metastatic infections may lead to relapsing bacteremia or poor outcomes. To determine predictive factors for metastatic infection of *S. aureus* bacteremia, we analyzed several factors, including the underlying disease, initial antimicrobial treatment, and primary site of infection. From January 2008 through December 2011, 40 patients met the inclusion criteria of this study. Of the cases of bacteremia in these patients, 33 were caused by methicillin-sensitive *S. aureus*. Metastatic infection occurred in 11 (27.5%) of the 40 patients. The predictive factors associated with the development of metastatic infection identified with multivariate analysis were unknown primary site of infection and fever persisting for more than 72 hours after the start of antibiotic treatment.

The capacity to form biofilms and the biofilm component of staphylococci

Staphylococci are able to attach to abiotic or biotic surfaces and form biofilms, which lead to chronic infections. We analyzed the capacity to form biofilms and the biofilm component *in vitro* of clinically isolated staphylococci. Biofilm formation in brain-heart infusion broth was similarly observed in 48 strains of *S. aureus* (29%) and 28 strains of *Staphylococcus epidermidis* (25%). One biofilm formed by *S. aureus* and 4 biofilms formed by *S. epidermidis* were susceptible to polysaccharide degradative enzyme (dispersin B). These results indicate that the biofilms formed by *S. epidermidis* contain more polysaccharide than do biofilms formed by *S. aureus*.

Clinical analysis of the immunological background of patients with nontuberculous mycobacteriosis

We are investigating the pathophysiology of infections in patients with nontuberculous

mycobacteriosis and correlating immunological variables with images of bronchopulmonary lesions.

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Department of Dentistry

Masashi Sugisaki, *Professor*
Katsuhiko Hayashi, *Associate Professor*

Akihiro Ikai, *Professor*
Shigeru Suzuki, *Assistant Professor*

General Summary

1. Clinical studies of temporomandibular disorders

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

2. Clinical studies of dentistry for medically compromised patients

We continued our studies of the usefulness of diagnostic equipment for medically compromised patients.

3. Clinical studies of obstructive sleep apnea-hypopnea syndrome

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

4. Basic studies of growth factors in the oral cavity

We investigated the expression patterns of nerve growth factor (NGF) and NGF receptors in human salivary glands and the secretion of NGF in human saliva.

Research Activities

Clinical studies of TMDs

1. Relationship between TMDs and job content in the employed population of the Tokyo metropolis

Purpose: We previously reported the prevalence of TMDs and factors contributing to TMDs in the employed population of the Tokyo metropolis, based on a survey using a TMD screening questionnaire. Significant factors contributing to TMD identified with multivariate logistic regression analysis were a feeling of fatigue (odds ratio [OR] = 1.55) in men and depression (OR = 1.37) and a feeling of fatigue (OR = 1.30) in women. The purpose of this pilot study was to investigate the sex-related differences in the relationship between job contents and TMDs.

Methods: In 2007 and 2009 we performed a questionnaire survey, with the cooperation of the Tokyo Dental Association, of subjects undergoing dental checks at general dental clinics in Tokyo. Responses were obtained from 244 subjects in 2007 and 76 subjects in 2009 and were used as secondary data for the analysis. We considered subjects who gave any answer other than "0" to the question of commuting time to be employed (256 subjects). The questionnaire included 4 TMD-screening items, sex, age, and 9 job-content items, and the responses were analyzed with multivariate logistic regression analysis.

Results: The mean age did not differ between men and women. In regard to sex differences in job contents, men had a significantly longer driving time, and women had a longer time at home before going to bed (Bonferroni correction value: $p = 0.0062$). The personal computer (PC) operation time did not differ between the sexes. Because multi-

variate logistic regression analysis identified sex as a significant factor ($OR = 0.417$), we examined the sex-related differences in the relationship between TMD and job contents. The PC operation time showed a significant OR (1.94) for TMD only in women.

Conclusion: Sex-related differences were suggested in the relationship between TMD and job contents. PC-related work was identified as a factor contributing to TMD in women.

2. Criterion-related validity of a questionnaire to assess pain-related limitation of daily functions of Japanese patients with TMDs

Purpose: Reliability and validity are required characteristics of a questionnaire. We devised a questionnaire to assess pain-related limitation of daily functions in Japanese patients with TMDs (LDF-TMDQ) and reported its construct validity and reliability in 2005. In addition, we created a TMD-screening questionnaire and reported its validity in 2007. The purpose of this study was to assess the concurrent validity of the LDF-TMDQ.

Methods: This study was performed as a part of a multicenter joint investigation for a pluralistic evaluation and the effect of the pluralistic treatment on patients with TMD. The 752 subjects used which the existing data as a second document with no missing values at the outpatient clinic of The Jikei University Department of Dentistry from October 2005. We used a specimen LDF-TMDQ and the specimen that I examined of the TMD screening questionnaire for statistical analysis. We used the specimen is different from the specimen which statistical examined of LDF-TMDQ and TMD screening questionnaire. The TMD screening questionnaire was used as an external criterion. The concurrent validity was evaluated with Spearman's correlation coefficient.

Results: The TMD screening questionnaire had a sensitivity of 85.9% and a specificity of 78.1%. Spearman's correlation coefficient was 0.624 ($p < 0.001$).

Conclusion: These results support the concurrent validity of the LDF-TMDQ.

3. Simple usage of screening test, quality of life assessment, and a treatment procedure for patients with TMDs

Because TMDs are usually diagnosed by exclusion, knowledge of all diseases manifesting as trismus (persistent contraction of the masseter) and as masticatory disorders is required. However, because dentists treat many patients every day, they might overlook 5 important symptoms for differential diagnosis: nervous system deficiency, a history of trauma, and clinical history taking, an oral aperture of less than 20 mm, and pain in the resting position. Because the understanding of the concept of "pain in the resting position" can differ between dentists and patients, the concept must be explained in plain words that the patient can understand. In a medical interview, confirmation of daily and diurnal variation of symptoms, characteristics of pain (act on onset and time of onset), job contents, sleep condition, and exercise contents are important. Furthermore, behavioral methods of correcting malocclusion, dietary counseling, sleep guidance, monitoring PC use time, mouth-opening exercises, and stress-relieving methods were explained. Advice to healthcare providers on pressure-pain exercises using 2.0 kg of finger pressure, stretch and massage methods in daily life, walking methods, and postural guidance are also important.

There is no treatment without a goal, and function improvements, such as attaining a painless oral aperture of 40 mm and chewing gum for 15 minutes, were suggested treat-

ment goals. Physicians must explain to the patient that the disease is not expressed as a final consultation, but self-care.

Study of dentistry for medically compromised patients

1. Clinical analysis of prothrombin time kits CoaguChek®XS and INRatio®2

Purpose: In our department, the CoaguChek®XS system (F. Hoffmann-La Roche, Ltd., Basel, Switzerland) has been used as a preoperative examination of prothrombin time (PT) for patients taking warfarin potassium. Because the CoaguChek®XS reagent is expensive, we examined whether prothrombin might instead be measured with the INRatio®2 system (Alere, Inc., Waltham, MA, USA). The aim of this study was to compare the PT/international normalized ratio (INR) results measured with CoaguChek®XS and INRatio®2 and to examine the relationship between the 2 systems.

Materials and Methods: Fifty-one patients (31 men and 20 women; mean age, 68 ± 13.9 years) were included in this retrospective study. A single finger puncture provided the retrospective data for 2 examinations; that is, the INR was measured with CoaguChek®XS and INRatio®2 at the same time. Although the INRatio®2 data were not normally distributed, the CoaguChek®XS data were normally distributed. For these reasons, statistical analysis involved paired *t*-tests, linear regression analyses, and determinations of effect size and a sample size.

Results: The mean INR values did not differ between the 2 systems. The adjusted R^2 of linear regression was 0.818. The effect size determined with the paired *t*-test was 0.130, which is small. The sample size was 324.

Discussion: Our results suggest that significance can be clarified with a larger sample size. In addition, each member of our department reported that INRatio®2 system required a much larger volume of blood and was more complicated to operate than the CoaguChek®XS system. Although both systems have an equal number of medical remuneration points, the INRatio®2 reagent is less expensive than the CoaguChek®XS reagent.

Consideration: This study did not demonstrate clear differences between the CoaguChek®XS and INRatio®2 systems. We finally decided to continue using the CoaguChek®XS system in our department.

Clinical studies of OSA-hypopnea syndrome

1. BMI and fatty change of the suprahyoid muscles

Purpose: A change in muscle function has been postulated to be associated with the etiology of OSA. Saito et al. have previously reported the effect of obesity on the properties of the lingual muscles (genioglossus and geniohyoid muscles to fat in rats. (*Arch Oral Biol* 2010; 55:803-808). However, such a muscle metamorphosis has not been shown in humans. Here, we show evidence of fatty metamorphosis in the lingual muscles in computed tomography (CT) images of patients suspected to have OSA.

Materials and Methods: The subjects were 66 patients (51 men and 15 women) suspected to have OSA who visited the Tsurumi University School of Dental Medicine from November 2007 through October 2011. All subjects gave informed consent to take part in the study. Subjects underwent CT (Radix Prima scanner, Hitachi Medical Co., Tokyo, Japan) evaluations at the image diagnosis department of the hospital. The tube voltage

used was 120 kV, and the tube current was 50 mA. Exposure time was 1 second. Sex, age, BMI, and apnea-hypopnea index (AHI) were recorded for each patient. The degree of fat-to-muscle metamorphosis was measured with CT. Image-analysis software (Aze Win[®], AZE, Japan, Tokyo) was used to set the region of interest (ROI) of 30 mm² on the bellies of the lingual muscles. We measured CT levels of 4 ROIs (both sides of the central area and both sides of the posterior area) in the genioglossus muscles and 2 sizes of ROI (both sides of the central area) in the geniohyoid muscles. Values were quantified and compared statistically.

Results: The median values (25% and 75% quartile deviations) were patient's age: 50.00 years old (42 and 61 years); BMI: 24.00 kg/m² (22.00 and 26.50 kg/m²); genioglossus CT levels: 121.55 (88.85 and 135.28), and geniohyoid muscle CT levels: 111.95 (104.28 and 116.25). The results of a multiple regression model were analyzed with the Amos (version 6) software package (Amos Development Corporation, Spring House, PA, USA); the standardized estimates of the BMI were -0.53 ($p = 0.000$) for the genioglossus muscle and -0.40 ($p = 0.002$) for the geniohyoid muscle.

Conclusion: Consistent with the report of Saito et al, we found evidence of fatty metamorphosis of the lingual muscles of humans with effects of the BMI. We are planning to investigate the effect of fatty changes of the lingual muscles on OSA.

Basic studies of growth factors in oral cavity

1. Distribution of NGF and its receptors in human salivary glands

Purpose: NGF is a pluripotent mediator present in a range of human tissues. The aim of this study was to investigate the expression pattern of NGF and NGF receptors in human salivary glands and the secretion of NGF in human saliva.

Methods: The distribution of NGF- β , proNGF, and their receptors tyrosine kinase receptor A (TrkA) and p75 neurotrophin receptor (p75^{NTR}) was examined qualitatively in human parotid ($n = 6$), submandibular ($n = 3$), sublingual ($n = 3$), and minor salivary glands ($n = 6$) with immunohistochemical methods. Western blotting of unstimulated human whole saliva ($n = 6$) with antibodies reactive with mature NGF- β or proNGF was performed to identify NGF forms.

Results: Intercalated, striated, and collecting ducts in all gland types showed strong staining for proNGF and weak staining for NGF- β in the submandibular, sublingual, and minor salivary glands. TrkA was also strongly expressed in the ducts of all gland types, whereas p75^{NTR} expression was confined mainly to collecting ducts. In acini, no or only weak cytoplasmic staining was seen for any of the markers. Western blotting of saliva showed secretion of several forms of proNGF, but no mature NGF- β was detected.

Conclusion: The ligand proNGF and the receptor TrkA might regulate the functions of duct cells, such as the release of signaling molecules, in an autocrine, paracrine, or solinocrine fashion. Secreted into the oral cavity, salivary proNGF can participate in normal wound healing, as it has been shown to increase the proliferation and migration of oral keratinocytes.

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Department of Transfusion Medicine

Tetsunori Tasaki, *Professor*

Yoko Kato, *Assistant Professor*

General Summary

1. Transfusion-related acute lung injury

Transfusion-related acute lung injury (TRALI) is characterized by the sudden onset of respiratory distress following blood transfusion and is considered one of the most serious complications of blood transfusion. On the other hand, similar adverse symptoms are sometimes observed in cardiac pulmonary edema due to massive or rapid transfusion of blood products, and this response is known as transfusion-associated circulatory overload (TACO). Although TRALI and TACO are both important problems of transfusion medicine, their risks are not fully recognized among physicians, and distinguishing the 2 conditions is occasionally difficult. With this background, a new study group (T. Tasaki, principal investigator) was formed by the Health and Labour Science Research Grants to establish guidelines that could help distinguish TRALI from TACO and guide subsequent treatment.

2. The risk of bacterial contamination in blood derived from volunteer donors having loose bowels

In Japan, the main reason for the reduction in the shelf life of red cell components from 6 weeks to 3 weeks was the risk of contamination by bacteria, such as *Yersinia enterocolitica*, that can grow at low temperatures (4°C). On the other hand, the demand for blood usage in Japan has gradually increased because of the aging population and the low birth rate. Therefore, the possibility of prolonging the storage period has been investigated from the points of view of the risk of bacterial contamination and the cost benefits. This study was supported by Grants-in Aid for Scientific Research.

3. Pediatric transfusion

There remain unresolved problems in pediatric transfusions. Hemolysis due to blood transfusion with long, narrow tubes is a concern for very low birth weight infants. Recently, a new transfusion device with a peristaltic roller system that moves blood through the tube without damaging blood cells has been introduced for such cases. We investigated the advantages of the new device for preventing hemolysis.

4. A case with anti-Kp^c antibodies

Research Activities

1. In the first year of research on TRALI and TACO, the following topics were investigated: 1) recent transfusion-related adverse events around the world related to TRALI and TACO, 2) the relationship between anti-leukocyte antibodies in the donor blood and dyspnea in recipients, 3) trends in perioperative transfusion of blood components including albumin, and 4) experimental massive transfusion using animal models. In nearly 30% of patients suspected of having TRALI, dyspnea appeared to be due to

TACO. Recently, an average of 2 patients die of TRALI each year in Japan. In more than 95% of cases with an intraoperative blood loss of more than 1,000 mL, albumin was used. However, albumin was used in about 60% of cases with less blood loss. Studies of adverse events due to donors' anti-leukocyte antibodies and studies to establish animal models of TACO are now underway.

2. In the hundreds of blood specimens, including those from donors having loose bowels, blood cultures did not show bacterial contamination. The results suggest that the risk of bacterial contamination is extremely low. In our hospital, the main reason (> 40% of cases) for the disposal of packed red cells was that the product had exceeded its shelf life. These facts support the extension of the storage period from 3 weeks to 6 weeks. However, the new data would likely support the longer storage period in Japan only during a serious shortage of blood, due, for example, to a pandemic or earthquake.

3. Contrary to our expectation, the rate of hemolysis with the new device was slightly greater than that with the currently used syringe infusion pump, although the difference was small. Transfusion of newer blood using a potassium-reduction filter would be preferable for very low birth weight infants. This result was reported at the 60th meeting of the Japan Society of Transfusion Medicine and Cell Therapy.

4. We reported an extremely rare case of alloantibodies (anti-Kp^c) against very low incidence (<0.01%) red blood cell antigens. The patient received 2 units of incompatible blood and developed shock due to a delayed hemolytic transfusion reaction but recovered within 10 days without prolonged hemolytic complications. This case was reported at the 60th meeting of the Japan Society of Transfusion Medicine and Cell Therapy.

Publications

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Institute of DNA Medicine

Department of Gene Therapy

Toya Ohashi, *Professor and Director*

Hiroshi Kobayashi, *Associate Professor*

General Summary

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

Research Activities

Novel BMT methods for LSDs

Many LSDs are treated with BMT. However, preconditioning regimens with high-dose chemotherapy and high-dose irradiation worsen the general condition of patients. Thus, we have been developing a safer preconditioning regimen. Hematopoietic stem cells (HSCs) in recipients of BMT must be killed by preconditioning. However, most HSCs are not dividing and are resistant to chemotherapy and radiation. We hypothesized that if HSCs were dividing, the doses of chemotherapy and irradiation could be reduced. We focused on bone marrow niche, inducer of type 1 interferon, and anti-cKit antibody were used for preconditioning for BMT in mucopolysaccharidosis mice. As a result, engraftment of a sufficient number of donor cells was achieved and exhibited a therapeutic effect without significant side effects.

Immune tolerance induction of ERT for LSDs

ERT is extremely effective for Pompe disease. However, its efficacy is decreased by antibodies against infused enzymes. We have previously shown that oral administration of an enzyme induced immune tolerance against the enzyme (Ohashi T, et al. *Mol Genet Metab*, 2011). In this study we immunized mice with intraperitoneal injections of an enzyme and adjuvant. To assess the possible clinical application of this strategy, we immunized mice with an infusion regimen similar to the clinical protocol with biweekly intravenous infusion of enzymes (20 mg/kg). In this immunization protocol, oral administration of enzyme reduced the titer of antibodies against the enzyme and prevented lethal allergic reaction to the enzyme.

Gene therapy for LSDs

We studied the therapeutic effect of gene therapy in a neonatal mouse model of Krabbe disease, a progressive demyelinating disease. We injected a recombinant lentiviral vec-

tor including the therapeutic gene expressing galactocerebrosidase (GALC) into the facial vein of neonatal mice and detected significant effects, including reduced substrate accumulation, decreased pathological findings in Schwann cells, delayed symptom onset, and increased life span. We also investigated neonatal gene therapy for mucopolysaccharidosis (MPS) type VII (Sly disease) which resulted in efficient enzyme expression in the brain. We are attempting *ex vivo* gene therapy using a lentivirus system for MPS II (Hunter disease) and attempting to transduce cells from a patient with Pompe disease into induced pluripotent stem cells. We also studied the synergic effect with substrate reduction therapy and are preparing a homologous recombination system using Zinc-finger nuclease.

Novel therapy for Pompe disease with an enzyme-stabilizing agent

An ERT with recombinant human acid alpha-glucosidase (GAA) was recently approved for treating Pompe disease. This ERT prolongs survival and decreases cardiac muscle pathology, but has several problems, such as resistance in skeletal muscles and production of antibodies against recombinant human GAA. Therefore, an alternative method of addressing GAA deficiency is needed for the effective treatment of patients with Pompe disease. In this study, we focused on the pathogenic mechanism by which mutant GAA is degraded by endoplasmic reticulum-associated protein degradation in some patients with Pompe disease and investigated whether 2 proteasome inhibitors (bortezomib and MG132) restore the function of mutant GAA in fibroblasts from patient with Pompe disease. Each proteasome inhibitor promoted the stabilization of patient GAA and the processing of them to mature forms at any concentration tested. In addition, lower concentrations of bortezomib and MG132 showed no cytotoxic effects in patient fibroblasts. Increased colocalization of GAA with the lysosomal marker lysosome-associated membrane protein 2 were observed in patient fibroblasts treated with proteasome inhibitors. Furthermore, proteasome inhibitors also increased enzyme activity in patient fibroblasts. In particular, bortezomib was more effective than MG132 in enhancing GAA activity in patient fibroblasts (about 4-fold and 2-fold increases of residual activity, respectively). These results suggest that bortezomib is a novel drug for treating patients with Pompe disease.

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

Recent studies have demonstrated that nuclear factor (NF)- κ B plays important roles in the regulation of cell apoptosis, inflammation, and oncogenesis. Inhibition of NF- κ B is a potential new strategy for the treatment of cancers. We have previously reported that nafamostat mesilate, a serine-protease inhibitor, inhibits NF- κ B activation and induces the apoptosis of pancreatic cancer. Moreover, we have shown that the addition of nafamostat mesilate promotes apoptosis induced by gemcitabine or paclitaxel owing to the inhibition of the NF- κ B activation of pancreatic, gastric, and gallbladder cancers. The clinical usefulness of the combination of gemcitabine and nafamostat mesilate for patients with unresectable pancreatic cancer was examined in a phase II study. Recently we have investigated the antitumor efficacy of combination therapy with nafamostat mesilate and

radiation for pancreatic cancer.

Recent studies have found that human CD40 ligand (CD40L) gene delivery has direct antitumor effect via CD40-CD40L interaction. However, CD40L enhances activation of NF- κ B. We have previously reported that nafamostat mesilate inhibits NF- κ B activation and enhances apoptosis caused by adenovirus vector-mediated tumor necrosis factor α in pancreatic and hepatocellular carcinoma. Therefore, we have investigated the efficacy of combination therapy with nafamostat mesilate and adenovirus vector-mediated CD40L gene therapy for pancreatic cancer.

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Institute of DNA Medicine

Department of Oncology

Sadamu Homma, *Professor and Director*
Shigeo Kido, *Associate Professor*

Mikio Zeniya, *Professor*
Yasuharu Akasaki, *Assistant Professor*

General Summary

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

Research Activities

A phase I clinical study of immunotherapy against advanced pancreatic cancer using dendritic cells pulsed with Wilm's tumor 1 class I and II peptide

The activation of helper T cells should be an essential factor to induce effective antitumor immunity. Vaccination with dendritic cells (DCs) pulsed with Wilm's tumor 1 (WT1) class I and II peptides would stimulate WT1-specific cytotoxic T cells, as well as helper T cells, leading to the induction of potent WT1-specific antitumor immunity. This clinical study is the first trial of treatment with DCs pulsed with WT1 class I and II peptides. A 64-year-old man with advanced pancreatic cancer has received this treatment and has been well in good shape for 10 months.

A phase I clinical study of immunotherapy against glioblastoma using a DC/tumor fusion cell vaccine

The survival of patients with glioblastoma for more than 4 years has been achieved with the combined treatment with temozolomide and a DC-vaccine generated by the fusion of autologous DCs and irradiated glioblastoma cells. The overall survival of patients with glioblastoma treated with the both temozolomide and DCs was significantly longer than that of patients treated with temozolomide alone.

Generation of an artificial protein vaccine inducing potent cellular immunity

Artificial proteins composed of a cytotoxic T-lymphocyte (CTL) epitopes, helper epitopes, and intercalated peptides of ovalbumin (OVA) were generated using the MolCraft system for protein evolution. Vaccination of mice with the protein of the most proper peptide arrangement demonstrated strong induction of OVA-specific CTLs and elicited the inhibition of OVA-expressing tumor growth. MolCraft-generated artificial proteins are promising future cancer vaccines.

Identification of unique cancer-associated molecules as candidates for novel cancer vaccines

HLA class I binding peptides were collected from cultured human prostate cancer cells and analyzed with liquid chromatography/tandem mass spectrometry. Several peptides derived from unique cancer-associated proteins were identified. The peptides of absent in melanoma 1-like protein (AIM1L), transmembrane protein-191C (TMEM191C), and c20orf201 were expressed in various types of cancer cell but only in testis, cerebellum, and placenta among noncancerous tissues. These peptides are promising candidates for novel cancer vaccines.

A new molecularly targeted therapy against pancreatic cancer based on the up-regulation of human epidermal growth factor receptor 2 expression

Human pancreatic cancer frequently expresses human epidermal growth factor receptor 2 (HER2). We found that treatment of human pancreatic cancer cells with gemcitabine enhanced HER2 expression. Trastuzumab emtansine (T-DM1), a unique conjugate of a monoclonal antibody to HER2 and a chemotherapeutic agent, showed strong cytotoxic activity against gemcitabine-pretreated pancreatic cancer cells. This synergistic effect might be closely associated with enhanced T-DM1 binding to HER2, the expression of which was up-regulated by treatment with gemcitabine.

Inhibition of the expression of the immune-suppressive protein programmed cell death-ligand 1 on cancer cells by chemotherapeutic agents or molecularly targeted agents or both

The CTLs attacking tumor cells would be killed by the interaction between programmed cell death-ligand 1 (PD-L1) on cancer cells and programmed cell death 1 (PD1) on CTLs. We found that some inhibitors of nuclear factor- κ B are able to reduce PD-L1 expression on cancer cells. Treatment with such agents might contribute to the inhibition of PD-L1-mediated immune suppression, leading to augmentation of the antitumor effect of cancer vaccines.

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Institute of DNA Medicine

Department of Molecular Genetics

Hisashi Yamada, *Professor and Director*

Takeshi Kawano, *Assistant Professor*

General Summary

The fates of most cells are genetically and epigenetically regulated. Therefore, we intend to study the pathogenesis and therapy of diseases based on this knowledge. The diseases we are interested in are hematological malignancies, pediatric cancers, spinal muscular atrophy, and Alzheimer's disease (AD). We believe that these intractable diseases will eventually be conquered through the harmonization of basic science and clinical medicine.

Research Activities

Cancer Molecular Biology

Leukemia consists of 2 types of cell. One type is rapidly growing mature leukemia cells, and the other type is slowly growing leukemia stem cells. Mature leukemia cells are highly sensitive to antileukemia drugs. In contrast, leukemic stem cells are resistant to drugs and remain alive during the ordinary induction therapy, resulting in the relapse of leukemia. However, the definition of leukemia stem cells is not concrete, and leukemic cells change their phenotype under the influence of their growth environment. We are studying the mechanism of leukemic cell plasticity using JAS-R megakaryocytic leukemia cells. We have found that hypoxia and cellular attachment to the environmental matrix are critical factors for leukemic cell plasticity.

Molecular pharmacology of anticancer agents

We are investigating the action of the following anticancer agents: telomerase inhibitors, histone deacetylase inhibitors, tyrosine kinase inhibitors, and DNA topoisomerase I inhibitors. In addition, we are studying the molecular mechanism of new agents called bromodomain inhibitors. Bromodomain proteins link acetylated histones to a set of transcriptional machinery proteins that are important for initiating cell proliferation. Bromodomain inhibitors inhibit proliferation of a small number of cancers. However, why only a few cancers are vulnerable to the inhibitors remains unclear. Bromodomain inhibitors and other epigenetic modifiers may be ideal anticancer agents, because of their new mechanisms of actions and because they do not directly damage DNA. These drugs will reduce the rate of therapy-related malignancies.

Molecular genetic approach to neurological diseases

The homozygous deletion and mutation of survival motor neuron (SMN) 1 gene causes the hereditary neurodegenerative disorder spinal muscular atrophy (SMA). A nearly identical gene, SMN2, also exists but cannot prevent SMA. We have previously shown

that a single-base difference in exon 7 of SMN2 creates a heterogeneous nuclear ribonucleoprotein (hnRNP) A1/A2-dependent exonic splicing silencer and that the specific knock-down of A1/A2 in cells effectively rescues exon 7 splicing in SMN2. However, we also found that A2-specific knock-down represses SMN synthesis.

The aim of this study was the molecular characterization of the above hnRNP A2-specific RNA interference-mediated SMN reduction and to find a molecular target as the basis of a new agent for treating SMA. We found that hnRNP A2 plays a significant role in the efficient translation of SMN2 messenger (m) RNA under ubiquitous conditions. Thus, the reduction of hnRNP A2 in cells decreases this activity, such that SMN is not efficiently translated. Additionally, we propose that the hnRNP A2-binding sequence at SMN 3'-UTR functions as a translational enhancer to efficiently activate translation from SMN1/2 mRNA. Our findings define a new regulatory mechanism to control SMN protein production, provide new insights into the function of cellular hnRNP A2 in translation, and suggest a pathway to new agents for treating SMA.

AD is a progressive, degenerative disease for which there is no cure. Both patients with amnesic mild cognitive impairment and patients with mild AD show memory impairment and executive dysfunction as core symptoms. Moreover, some cases of amnesic mild cognitive impairment will eventually progress to AD. We are investigating the differences between mild AD and amnesic mild cognitive impairment by studying the single nucleotide polymorphism of brain-derived neurotrophic factor and nerve growth factor. The *BDNF* gene may significantly influence the executive function of mild AD.

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Institute of DNA Medicine

Department of Molecular Immunology

Saburo Saito, Associate Professor and Director
Nobutake Akiyama, Assistant Professor

Daitaro Kurosaka, Associate Professor
Yuji Ohno, Assistant Professor

General Summary

Our research interests have focused on the analysis of the basic immune system, which protects us from a number of diseases, and of immune disorders, such as hypersensitivity diseases and autoimmune diseases.

Research Activities

Pleiotropic function of interleukin 31

Interleukin (IL) 31 is a T-cell-derived cytokine that induces severe pruritus, hair loss, and dermatitis and is involved in allergic diseases, such as atopic dermatitis and bronchitis. To investigate the function of IL-31, IL-31 transgenic mice were created in our laboratory. In addition to scratching behavior and hair loss as reported previously, enhancement of the serum immunoglobulin (Ig) E level was observed in the IL-31 transgenic mice. Moreover, these pleiotropic functions were verified by the administration of IL-31 into normal mice.

Furthermore, to investigate the function and the locations of IL-31 or the IL-31 receptor, 2 strains of IL-31 or IL-31 receptor (IL-31R) knockout/*LacZ* knockin mice were generated. In *IL-31R^{+/lacZ}* knockin mice 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside staining was limited to the hair matrix. To produce offspring with a genetic identity for the analysis of the pleiotropic functions of IL-31, the heterozygous mouse will be backcrossed 6 or more times into the C57B/6J genetic background.

We tested the ability of *IL-31R^{lacZ/lacZ}* knockin mice to respond to IL-31 delivered via intradermal injection (10 μ g per day for 14 days) compared with the responses of heterozygous littermate control mice. Thus, neither alopecia nor pruritus developed in *IL-31R^{lacZ/lacZ}* knockin mice in response to IL-31 over the 14-day course of treatment.

A rice-based edible vaccine expressing Japanese cedar pollen allergens induces oral tolerance in Japanese monkeys with Japanese cedar pollinosis

Japanese cedar (*Cryptomeria japonica* [CJ]) pollinosis affects more than 30% of the Japanese population and is, thus, one of the most common diseases in Japan. Furthermore, CJ pollinosis has been found to occur naturally in Japanese macaques (*Macaca fuscata*), which show symptoms similar to those of human patients. Plants have recently been recognized as a form of bioreactor for the cost-effective production of large-scale recombinant proteins. The edible tissue of plants provides the further significant benefit of being a simple method of mucosal delivery of vaccines without the need for complicated purification steps.

Our previous study showed that oral administration to mice of transgenic rice seeds that have accumulated high concentrations of polypeptides derived from CJ pollen allergens reduces the mice's serum IgE levels and T-cell proliferative responses to CJ allergens, proving the efficacy of oral immunotherapy for the treatment of pollinosis.

In this study, the transgenic rice plants that had accumulated high concentrations of JC allergens were used for oral immunotherapy for CJ pollinosis in macaques. Five macaques with CJ pollinosis were fed once a day with 20 g of the rice seeds containing about 50 to 60 mg of allergens for 3 months. No side effects, such as urticaria, dyspnea, vomiting, and weight loss, were observed during immunotherapy. One and a half months after the start of feeding, proliferative responses of T cells to JC allergens in 4 of 5 macaques were significantly inhibited compared with those in macaques at the start of feeding. However, their T-cell responses to CJ allergens were restored 1 month after the end of feeding. On the other hand, in healthy macaques without CJ pollinosis, the side effects and the induction of immune responses to CJ allergens were not observed after oral administration of transgenic rice seeds.

These results indicate that oral immunotherapy with transgenic rice seeds is a safe and effective treatment for pollinosis.

Induction of cytotoxic T lymphocytes with liposome-based adjuvants

Vaccines that induce specific cytotoxic T lymphocytes (CTLs) against tumors and pathogens appear to be a promising approach to treating these diseases. To make efficient T-cell vaccines, several types of liposome were developed to induce CTLs specific to antigens. For the clinical use of these vaccines, the absorption of antigen into liposome and the stabilization of these complexes are always troublesome.

To overcome these problems, we have developed liposome-based synthetic adjuvants, which enable absorption when mixed with antigens just before injection, and analyzed the properties of the adjuvants with chicken ovalbumin (OVA) as a model antigen. The OVA-specific CTLs were efficiently induced within 4 days after a single immunization and were maintained for more than 2 weeks. The induced CTLs were antigen dose-dependent and showed more than 95% killing activity with an *in vitro* CTL assay. These CTL activities were also confirmed with an *in vitro* chromium-release assay and with tumor growth inhibition *in vivo*.

Publications

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Institute of DNA Medicine

Department of Molecular Cell Biology

Yoshinobu Manome, *Professor*

General Summary

The department provides molecular tools for analyzing the medical problems under both physiological and pathological conditions. To elucidate the underlying mechanisms of such problems, molecular biological technologies using cells are useful. The methods include modification of nucleic acid transcription and expression by transfection of DNA or short interfering RNA. Also, labeling of molecules with fluorescent nanoparticles, conjugation to sensors, and amplification with radiolabelled materials are used. By introducing such methods of molecular and cellular biology, we are helping address clinical problems.

Research Activities

Development of a nucleic acid delivery system for malignant glioma cells by acoustic energy

Glioma is an intractable disease of the central nervous system. Because the prognosis is poor, alternative therapies are required. Despite the poor prognosis, metastasis outside the central nervous system is rare, and the cause of the death in most cases is local recurrence. Therefore, if an effective local therapy were established, patients would live longer, and even complete cure could be expected. Recently, local therapy using a therapeutic ultrasound irradiator was developed, and the beneficial effect of therapeutic insonation to glioma in combination with microbubbles was reported. In the present study, to enhance the therapeutic efficacy, a nucleic acid delivery system using ultrasound was developed. Ultrasound conditions for delivery as well as adjuvant factors and transcripts of target genes were examined. Also, the effects of ultrasound for gene transcriptions were examined.

Transcription of urocortin and corticotropin-releasing factors (CRFs) in human malignant glioma cells

Urocortin (Ucn) and corticotropin-releasing factors (CRFs) and their receptors are expressed in many organs, including the central nervous system. This year, the expression of the mRNAs of Ucn I, II, and III and CRF and CRF receptors 1 and 2 in malignant glioma was examined. The RNAs of 5 human and 3 rat glioma cell lines were isolated, and transcripts in these cells were analyzed on the basis of complementary DNAs. Human and rat cell transcripts of Ucn and CRF receptors were detected in human glioma cells. When human KNS42 cells were exposed to proliferative and cytotoxic stimulation, transcription altered according to the conditions. However, although the quantities of transcripts varied with the proliferative and cytotoxic stimulation, the

overall transcription pattern was not affected by these stimuli.

Possible involvement of Ucn I in the adaptation to oxidative stress in HL-1 cardiomyocytes

Our previous studies have revealed that Ucn I is regulated by oxidative stress and that Ucn I suppress oxidative stress in a murine atrial cell line, HL-1 cardiomyocytes. Therefore, we investigated the involvement of Ucn I in the suppressive action in HL-1 cardiomyocytes. Ucn I was knocked down by small interfering RNA, and knock-down of Ucn I in HL-1 cardiomyocytes resulted in enhancement of nicotine-induced oxidative stress. In addition, investigation of the effects of nicotine on promoter activity using an Ucn I promoter-driven reporter plasmid revealed that nicotine enhanced the promoter activity of Ucn I. These results may contribute to the future development of cardioprotective strategies for cardiac disease.

Carbohydrate analyses of thyroid carcinoma cell lines using lectins

In our previous studies, we have reported a potential marker for thyroid papillary carcinoma, sialylated fibronectin antigen, which was detected with “JT antibody,” and developed applications for it. However, the details of the carbohydrate structure of the antigen in thyroid carcinoma remain unclear. Therefore, we investigated the structure of 5 thyroid carcinoma cell lines (K1 and IHH4 papillary carcinoma cell lines and SW1736, 8305C, and 8505C anaplastic carcinoma cell lines) using several lectins, which recognize specific carbohydrate structures. This research showed that SW1736 and IHH4 cells, which have high invasive capacity, were predominantly recognized by the lectin from *Datura stramonium*. Because *D. stramonium* lectin recognizes Gal β 1-4GlcNAc carbohydrates, these cells could express these structures. The next step would be to determine the carbohydrate structures of the fibronectin antigen in thyroid carcinoma cell lines.

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Institute of DNA Medicine

Project Laboratory for Kidney Regeneration

Takashi Yokoo, *Assistant Professor and Director*

General Summary

Recent advances in stem cell research have brought the possible use of somatic stem cells for organ regeneration one step closer to realization. Newly developed organs can then be used in clinical organ replacement. However, anatomically complicated organs, such as the kidney, have proven more refractory to stem cell-based regenerative techniques.

The kidney retains the potential to regenerate if the damage is not too severe and if the kidney structure remains intact. However, in cases of irreversible damage to the kidney, as can occur with long-term dialysis, the property of self-renewal is lost. Thus, any application of regenerative medicine in chronic renal disease will require the *de novo* development of an entire functional kidney.

It was previously believed that bone marrow-derived stem cells could differentiate into renal-resident cells and participate in kidney regeneration after renal ischemia/reperfusion injury; however, recent studies suggest that the number of bone marrow-derived cells that engraft injured tubules and develop into functional renal tissue is extremely small and that, therefore, their overall contribution to renal repair would be minor. For this reason we are attempting to develop entire kidneys using more premature and pluripotent stem cells.

We have used the developmental program of a developing organ by transplanting it into an ectopic site where it continued to develop *in vivo*. This procedure would facilitate the inward migration of autologous stem cells that would then be stimulated by the developmental program of the xeno-organ to mature into tissue-specific cells.

Research Activities

This year, we determined the metabolic function of transplanted metanephroi with particular reference to renin production and effect on blood pressure. Ten-week-old male Wistar rats were divided into 4 groups: a control group ($n = 12$), a para-aorta-transplanted group ($n = 12$), an omentum/epididymis-transplanted group ($n = 14$), and a nontransplanted group ($n = 16$). Rats in both transplanted groups underwent metanephros transplantation and bilateral nephrectomy. Rats in the other groups underwent bilateral nephrectomy only. Two weeks later, rats in the transplanted and nontransplanted groups underwent residual nephrectomy, and hypotension was induced through intravenous infusion of diltiazem hydrochloride or rapid withdrawal of blood. Mean arterial blood pressure was monitored from the left femoral artery. Plasma renin activity was analyzed at multiple time points. The renin expression of transplanted metanephroi was evaluated with the real-time polymerase chain reaction and immunopathologically when the rats were killed. Metanephros transplantation significantly increased plasma renin activity

and maintained mean arterial pressure compared with those in the nontransplanted group. No significant differences between the para-aorta-transplanted, omentum/epididymis-transplanted, and control groups were found with respect to plasma renin activity and mean arterial pressure. The present study has shown that transplantation of metanephroi increases plasma renin activity and contributes to the control of blood pressure variability in hypotensive rats.

Publications

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Department of Neuroscience Laboratory of Neurophysiology

Fusao Kato, *Professor and Director*

Ayako M. Watabe, *Assistant Professor*

General Summary

The integration and coordination of functions throughout the body is realized mainly through intercommunication via the nervous systems. To understand how the activities of organs affect brain activity and, in turn, how the brain controls the activities of organs to optimize these integrative functions, we must clarify the mechanisms underlying the dynamic cell-to-cell signaling in the central nervous system (CNS) underlying various specific functions, such as autonomic regulation and pain sensation. In particular, plastic changes of the CNS “wiring” realized through the variability of synaptic connections in response to various environmental changes form the core mechanism for optimizing human and animal behaviors. We use approaches at the molecular, cellular, and network levels, including the patch-clamp recording of synaptic currents, the real-time imaging of the intracellular Ca^{2+} concentration, and optogenetic approaches to activate a specific set of neurons by light, in living brain tissues from normal animals, animal models of various types of disease, and animals subjected to experimental manipulation of gene expression and combine them with the behavior of these animals.

Research Activities

Central mechanisms of pain-related negative emotion

Using a rat model of chronic neuropathic pain, we demonstrated that synaptic potentiation at the excitatory synapses between afferent fibers arising from the lateral parabrachial nucleus and neurons in the central nucleus of the amygdala, a structure playing the principal role in expression of emotional behaviors, involves structural consolidation. We also demonstrated that in the streptozocin-induced model of painful diabetes neuropathy, the synaptic potentiation in the amygdala is established selectively in the parabrachial-central amygdalar synapses, unlike in other models. This finding further confirms the notion that the mechanism of synaptic potentiation in the central amygdala, which underlies the enhanced link between nociception and negative emotions, depends largely on the modality and duration of chronic pain.

Synaptic mechanism underlying acquisition and extinction of fear memory

The Pavlovian fear-conditioning paradigm depends on the association between a contiguously applied cue (e.g., tone) and an aversive signal (e.g., electric shock). It has been unequivocally established that the plasticity in the amygdala network plays the primary role in this associative learning. However, the origin and pathway of the aversive signal in fear conditioning have been only poorly identified. We hypothesized that the spino-parabrachio-amygdaloid pathway, which carries information arising from the nocicep-

tion-specific neurons in the spinal dorsal horn to the central amygdala by way of pontine parabrachial relay nucleus, would be one of the pathways for such aversive signals. We have shown that fear learning not only potentiates the indirect nociceptive pathway (basolateral amygdala to the central amygdala) but also the direct nociceptive pathway (parabrachial to the central amygdala). This finding is the first to demonstrate direct involvement of the plasticity in the spino-parabrachio-amygdaloid pathway in fear-learning.

Glia-neuron interaction at central synapses

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

Publications

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Institute for High Dimensional Medical Imaging

Naoki Suzuki, *Professor*

Asaki Hattori, *Associate Professor*

General Summary

The goal of our research is to develop new imaging systems that can be applied to clinical medicine now and in the future. High-dimensional, i.e., three-dimensional (3D) and four-dimensional (4D), imaging techniques have enabled noninvasive, realistic, uninhibited, and accurate observations of human spatial structures and their dynamics. The availability of real-time imaging using high-performance computers and medical virtual reality systems has expanded the possibilities for diagnosis, treatment, surgery, and medical education. The Institute for High Dimensional Medical Imaging has, therefore, established a system that facilitates cooperative research and development with international researchers and organizations.

Research Activities

Clinical application of high-definition, real-time medical imaging

We are performing research on the development of medical high-definition imaging technology and its clinical application using functional and morphological data obtained with X-ray computed tomography (CT) and magnetic resonance imaging.

We are developing a 4D motion system for analyzing human activities, such as the motions of the whole body. The system is driven by motion data obtained from anatomical and skeletal muscle models reconstructed from X-ray CT data sets.

This research is being performed by departments in our university in collaboration with Osaka University and Mayo Clinic (Rochester, MN, USA).

Development of endoscopic surgical robot system

We are developing an endoscopic surgical robot system that can be used to perform natural orifice transluminal endoscopic surgery (NOTES). Robotic instruments enter the abdominal cavity orally and are used to perform surgery on the abdominal organs.

This year we focused on developing a multiview camera system and other surgical instruments for surgical navigation during endoscopic and robotic surgery. This research was derived from the “Endoscopic Surgery Support System Research Development Project,” which we completed last year.

Development of Surgical Simulator for Various Surgical Techniques

Based on technology obtained from developing a surgical simulator for a surgical robot system last year, we are developing a simulator that can be used for various types of surgery, such as laparotomy and endoscopic surgeries.

This year, we created 3D models of surgical instruments needed for these surgeries and models of target organs and constructed an operational field environment in the virtual

reality environment.

Development of an image-guided surgery system

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

We are conducting joint research with clinical departments and developing systems that can be used for each type of surgery. This year we conducted numerous navigation surgeries: 5 cases with the Department of Surgery and 6 cases with the Department of Otorhinolaryngology in the operational ward in the high-tech navigation operating room of Daisan Hospital.

This year, we developed a method to display intraoperative registration needed for navigation to the surgeon more quickly and with greater accuracy. We also developed a method to display the names of organs in addition to the organ model on the navigation screen so that the surgeon is able to grasp the situation of the surgical field in a shorter period of time.

Moreover, we have started to develop a method to reflect organ deformation to the navigation system during surgery. The method we are developing measures the organ-surface configuration. The method reflects the change in the organ to the navigation by changing the organ model based on the measurement.

Application of high-definition medical image analysis to forensic medicine

By applying technology that we have developed for analyzing high-definition medical images, we are analyzing X-ray CT data sets of crime victims with the aim of developing new methods for future criminal investigations and for establishing new methods for creating court documents.

This year, by introducing stereoscopic views into the analysis system we enabled court juries to grasp the 3D configuration of a victim's injuries more intuitively. We developed a system that displays the 3D model of a suspected weapon, such as a knife, in the same 3D space as the victim's X-ray CT data set so that juries are able to easily recognize that the shape of the weapon matches the victim's injury.

As we did last year, this year we carried out 3D analyses of the position, depth, and angle of the attempted-murder victim's injuries using the victim's X-ray CT data set.

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

Publications

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Institute of Clinical Medicine and Research

Norio Tada, *Professor and Director*
Yoshihisa Namiki, *Associate Professor*

Akihito Tsubota, *Professor*

General Summary

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

Research Activities

Transporter gene in the treatment of chronic HCV infection

Combination therapy with pegylated interferon and ribavirin is the standard-of-care treatment for chronic infection with HCV. In this treatment, exposure of HCV to ribavirin in hepatocytes is critical for virus eradication. Ribavirin is transported into hepatocytes by cell-membrane transporters. We are investigating the function of the transporters and the association of single nucleotide polymorphisms of the gene with treatment response.

Comprehensive gene expression profiling analysis of microRNA/mRNA in liver tissue

We are profiling and analyzing the expression of microRNA/mRNA in the liver tissue of patients with chronic HCV infection who would receive standard-of-care treatment. We are analyzing whether the microRNA/mRNA candidates can be associated with treatment response in chronic HCV infection. When the candidates affect the treatment outcome, the function of the microRNA/mRNA will be investigated in detail.

The fabrication of “3D organic/inorganic-hybrid structure” as a future theranostic (therapy + diagnostic) and preventive nanomedicine

(Funding Program for Next Generation World-Leading Researchers ([JSPS])

(Funding for the Development of the Decontamination Technology [Ministry of the Environment: Government of Japan])

Free manipulation of the movement of drugs with remote-controlled light/magnetism/ultrasound used in cutting-edge medical technology is expected to be a next-generation technology. Remotely manipulating the speed and position of nanoparticles, which are

mineral capsules that respond to various types of physical energy and are filled with organic drugs, will lead to an innovative technology that allows “pinpoint” prevention, diagnosis, and treatment.

We aim to realize innovative nanomedicine in which we can remotely control the accumulation, release, and effects of drugs with nanosized capsules that efficiently convert light, magnetic, and ultrasonic energies. This is unprecedented research in which we can apply Japan’s world-leading nanotechnology to medicine. It will allow highly sensitive, rapid diagnosis and highly effective treatment that are gentle to the body for incurable diseases and for diseases that are difficult to diagnose. The realization of medical care that is gentle to the weak, such as elderly persons, will help promote a long and healthy life, reduce healthcare costs, and lead to the development of the healthcare industry. Moreover, because this technology can precisely control the behavior of drugs, it can be applied to diverse areas, such as pharmacology and biotechnology, agriculture, and environmental science.

Studies of lipid metabolism and atherosclerosis

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

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Division of Regenerative Medicine

Hiroataka James Okano, *Professor and Director*

General Summary

Induced pluripotent stem (iPS) cell technology has given us the ability to generate and expand various types of differentiated cell from patient-derived cells; iPS cells are now being applied to cell therapy and being used to study the mechanisms of disease. Our aim is to create experimental platforms using patient-derived iPS cells and genetically modified animal models suitable for research on human diseases. Recently, we have attempted to create a transgenic primate model of human neurodegenerative diseases through forced expression of dominant mutant genes. Advances in disease modeling with patient-derived cells and nonhuman primates will have an enormous effect on future opportunities and advances in biomedical research.

Research Activities

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

The Hu proteins (the neuronal Elav-like: nElavl) are the mammalian homologue of *Drosophila* Elav, an RNA-binding protein expressed in the nervous system. In the embryonic brain, Hu family proteins (HuB/C/D) induce neuronal differentiation by binding preferentially to GU-rich sequences with secondary binding to AU-rich sequences in target RNAs. HuC is widely expressed in all types of neuron and is persistently expressed from early embryonic development to adulthood. To study the function of HuC in mature neurons, we generated HuC-deficient knockout (HuC KO) mice. At 7 months of age, HuC KO mice exhibited intention tremor, gait abnormality, and ataxia. Before the onset of these symptoms, the axons of Purkinje cells underwent the morphological changes of swelling and retraction at the deep cerebellar nuclei, although the pathological changes were not observed during cerebellar development. Intriguingly, Purkinje cell soma seemed to be intact even at 21 months of age, and the number of the cells in HuC KO mice was the same as in their wild-type littermates. Histological analyses showed accumulation of mitochondria and amyloid precursor protein in the swollen Purkinje axons indicating that the axonal transport system might be impaired in HuC KO. To identify HuC targets in the adult cerebellum, we performed an RNA-binding protein immunoprecipitation-microarray assay and isolated several candidate RNAs, including Kinesin family members KIF3A and KIF3C, which are involved in axonal transport. Protein levels of KIF3A and 3C were significantly downregulated in HuC KO mice; however, their transcript levels were normal. Furthermore, protein production of KIF3A and 3C was induced without changing their transcript levels by co-expression of HuC with full-length KIF complementary DNAs in culture cells. These results indicate that HuC regulates the expression of KIF3A and 3C by a posttranscriptional mechanism *in vitro* and *in vivo*. The altered Kinesin expression could explain, at least in part, the

pathophysiological mechanisms of axonal degeneration in the cerebellum of HuC KO mice.

A transgenic nonhuman primate model of Parkinson's disease

Medical research based on animal models serves as a bridge between basic and clinical research. Among various experimental animals, a nonhuman primate model is of growing importance for research in neuroscience and related fields, including pharmacology, genetics, reproductive biology and social behavior. The common marmoset (*Callithrix jacchus*), a small new-world primate, is becoming increasingly popular in biomedical research, because of its advantage for translation to genetically close human systems. We used a lentiviral vector to generate a transgenic marmoset carrying a mutant form of human alpha-synuclein (SNCA). SNCA is a gene responsible for PARK1- and PARK3-type Parkinson's disease with an autosomal dominant pattern of inheritance. Lentivirus-transduced embryos were transferred to surrogate mothers, and 2 female founder animals were obtained. Each founder animal carried 2 copies of the transgene, and their expression was confirmed in blood and skin cells. The founders were periodically analyzed with minimally invasive methods, such as positron emission tomography and magnetic resonance imaging. Although positron emission tomography analysis using [¹¹C]PE2I indicated no significant difference between the transgenic and wild-type marmosets, magnetic resonance imaging analysis and voxel-based morphometry showed unilateral decreases in density in the substantia nigra in both transgenic animals, which might be an early sign of Parkinson's disease.

Advances in disease modeling using genetically modified primates will have huge impact on future opportunities and progress in the biomedical research on neurodegenerative diseases.

In-vivo imaging technology applied to regenerative medicine

The bioluminescent light technique has been broadly applied to the analysis of promoter activity in cells and the observation of internal structures in the animal body. A new chemiluminescent protein, firefly luciferase, enabled the visualization of extending neuronal growth cones and small protrusions on microglial cells in culture. Expression of firefly luciferase in transgenic mice allowed the video-rate time-lapse bioluminescent imaging of freely moving mice. By applying this *in vivo* imaging system to visualize cancer metastasis, we showed that a disintegrin and metalloproteinase 28 (ADAM28) cleaves von Willebrand factor and enhances metastasis by facilitating human carcinoma cells to escape from von Willebrand factor-induced apoptosis.

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Medical Engineering Laboratory

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

Hiroshi Furuhashi, *Professor*

General Summary

Medical engineering is an essential foundation for developments in medicine. In our laboratory, there are 2 key technologies: ultrasound and polymeric biomaterials. We have developed ultrasound technology for a new thrombolytic treatment for acute ischemic stroke. Our ultrasound research is characterized by the use of medium-frequency ultrasound and close collaborations with clinical departments and basic science departments, both in our university and hospital and others'. For the other key technology, polymeric biomaterials, we have applied these materials mainly for drug delivery systems. We have also recently applied polymeric biomaterials to imaging diagnosis through the synthesis of new polymeric contrast agents. In particular, we study polymeric micelle systems that can deliver both drugs and contrast agents. Therefore, these systems are called "theranostic" systems because of the dual functions of therapy and diagnostics.

Research Activities

Medical application of ultrasound

We have applied transcranial ultrasound therapy to the treatment of ischemic stroke. For this condition, injection of tissue plasminogen activator has been the only effective treatment. However, significant increases in therapeutic effects are desperately needed. Transcranial ultrasound can enhance the thrombolytic activity of tissue plasminogen activator. Our technology features the use of medium-frequency ultrasound, which enhances thrombolysis more than does ordinary ultrasound. However, medium-frequency ultrasound is believed to be associated with a high risk of brain hemorrhage. To minimize the risk of hemorrhage, we control both the period and interval of irradiation; we have shown in an animal model of high-blood-pressure brain ischemia (spontaneously hypertensive rat) that ultrasound irradiation is both safe and effective. Furthermore, we have found that modulation of the ultrasound reduced nonuniformity of the applied ultrasound. We have developed a new instrument that can rapidly determine the sonothrombolytic effect through the absorption of blood clots. We have been developing this new therapy through a "super special consortium for supporting the development of cutting-edge medical care" program supported by the Ministry of Health, Labour and Welfare of Japan.

Polymeric micelle drug carrier systems

Polymeric micelles are assemblies of synthetic polymers, which have been used for drug targeting. Professor Yokoyama, director of this laboratory, is an international pioneer in

the development of polymeric micelle targeting systems. Currently, 4 formulations of polymeric micelle anticancer drugs are undergoing clinical trials in Japan, Europe, and the United States. We are trying to establish the next-generation science technology in polymeric micelle systems. We are studying the polyethylene glycol (PEG)-related immune response, which is called the accelerated blood clearance (ABC) phenomenon, of the polymeric micelles. Curiously, the polymeric micelle carriers are very different from liposome systems. Although the surfaces of both carrier systems possess PEG, polymeric micelle carriers induced no or low immune responses of the ABC phenomenon. This avoidance of the immune response is a great advantage of polymeric micelle carrier systems for drug targeting. In this immune response, we have found that the generated anti-PEG antibody does not bind to the PEG main chain but does bind to the interface between PEG and hydrophobic blocks.

We are also performing basic chemistry studies to analyze the drug-incorporated inner core of polymeric micelles by use of synchrotron radiation (Super Photon Ring 8 Giga electron volt facility, Hyogo Prefecture). The precise measurement accurately determines the structure of polymeric micelles, and this knowledge can be used to better understand polymeric micelles in biological systems.

Polymer-based contrast agents for diagnosis

We have developed new polymeric micelle magnetic resonance imaging (MRI) contrast agents for the diagnosis of diseases. These contrast agents were proven to target sites of solid tumors and to exhibit clear MR images of extremely small tumors. Therefore, the polymeric micelles can be used for “theranostics” of tumors because the polymeric micelles can deliver both drugs and contrast agents to solid tumors. Furthermore, we are studying a novel application of the polymeric carrier system for diagnosing ischemic stroke. We observed that a polymeric micelle MRI contrast agent was successfully directed to a specific site in the ischemic hemisphere and provided high-contrast images that were not obtained with a conventional low molecular weight MRI contrast agent. This high contrast was obtained in a short time, such as 20 to 60 minutes, after the contrast agent was injected intravenously. Therefore, the polymeric micelle carrier system and polymer biomaterials may be extremely useful for both the diagnosis and treatment of ischemic stroke. This new challenge may lead to beneficial treatments.

Publications

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Division of Clinical Pharmacology and Therapeutics

Shigeru Kageyama, *Professor and Director*

General Summary

The Division of Clinical Pharmacology and Therapeutics was established in July 1995. The aim of the division is to investigate drug treatment, mainly in the area of internal medicine, whereas other departments of clinical pharmacology in Japan focus on registration trials, particularly phase I trials. Because a clinical laboratory where we had performed many human pharmacological studies became unavailable in 2003, we shifted our research from human studies to multicenter clinical trials and pharmacoepidemiological studies.

Research Activities

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

The above-mentioned statin study took a long time to complete. We organized a research group comprising academic and industrial organizations (Japanese Society for Pharmacoepidemiology, the Japanese Society of Clinical Pharmacology and Therapeutics, Japan Association for Medical Informatics, Japan Society of Clinical Trials and Research, the Federation of Pharmaceutical Manufacturer's Associations of Japan, Pharmaceutical Research and Manufacturers of America, and European Federation of Pharmaceutical Industries and Associations Japan) to make postmarketing studies more efficient by utilizing the Standardized Structured Medical-record Information eXchange (SS-MIX). The SS-MIX system was started in 2006 as a project supported by the Ministry of Health, Labour and Welfare for promoting the exchange of standardized medical information. The SS-MIX system will increase the efficiency of pharmacoepidemiological studies by identifying "new users" who started the drug after some period of nonuse. The "new user" design is often essential for unbiased results.

An administrative office for registration trials was established in the hospital in February 1999, and the system for registration trials in the hospital has been reformed to meet the demands of the new good clinical practice guidelines. Seven clinical research coordinators (CRCs) — 6 nurses and 1 pharmacist — now facilitate clinical trials. The CRCs have started to help with both registration trials and investigator-initiated trials. CRCs have been introduced into all registration trials since 2004; the quality and speed of these trials were much improved.

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

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Division of Molecular Epidemiology

Mitsuyoshi Urashima, Associate Professor and Director

General Summary

Despite having the same disease diagnosis, some patients may be cured but some may not. This difference cannot be understood with experimental medicine. On the other hand, clinical practice might also not provide the answer. We combined molecular biology and epidemiology to create the Division of Molecular Epidemiology, to clarify the etiology of disease and to predict factors affecting survival.

Research Activities

The Jikei clinical research course

From May 2012 through March 2013, we held 10 seminars about strategies for clinical studies for healthcare practitioners at The Jikei University. In 2012, small-group study courses targeting postgraduate students will be started from the principles of epidemiology and biostatistics by reading textbooks and by analyzing real clinical data with STATA software (StataCorp LP, College Station, TX, USA) and designing clinical studies. Our goal is for postgraduate students to develop the skills to construct hypotheses, design protocols, monitor trials, and analyze data.

Original studies

1. Randomized trial of vitamin D supplement
2. Genome and epigenome clinical studies and lead findings
3. Elective class of on global health

Publications

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Division of Clinical Epidemiology

Masato Matsushima, *Associate Professor and Director*

General Summary

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

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

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

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

Research Activities

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

Although little is known about the prognosis of patients receiving home medical care in Japan, few prospective cohort studies about elderly persons receiving home medical care have been performed in Japan. The EMPOWER-JAPAN study was started as a multi-center prospective cohort study to investigate in-home mortality and to clarify its predictors. The cohort consists of patients who are newly introduced to home medical care at more than 10 teaching clinics in Tokyo, Kanagawa, and Saitama. The follow-up period will be 4 years. This study is financially supported by the Japan Society for the Promotion of Science.

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

The chronic care model was developed during 1990s in the United States to improve the care of chronic illnesses by refining the care-provider system, especially in a primary-care setting. The aim of this research is to clarify the usefulness of the chronic care

model in Japan. The research plan consists of 2 steps. The first is to make an official Japanese version of the assessment form “Assessment of Chronic Illness Care (ACIC)” by following the World Health Organization procedure, for example, translation, back translation, and pilot study. This step has been finished. The second step is to compare the quality of diabetes care between specialists in diabetes and primary-care physicians as nonspecialists.

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

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

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Laboratory Animal Facilities

Hiroataka Kanuka, *Professor and Director*

Azumi Wada, *Assistant Professor*

General Summary

The purpose of the Laboratory Animal Facilities (LAF) is to support *in-vivo* research and to contribute to the development of basic and clinical medicine. In 2012, 179 researchers used the LAF. We undertake breeding of experimental animals and provide technical guidance to researchers in animal experimentation. In addition, we performed the following studies to develop basic medical sciences, including laboratory animal science.

Research Activities

Establishment and characterization of model animal for cancer research derived from Phodopus hamster

Our inbred strains derived from *Phodopus* hamsters are maintained in this laboratory. The *Phodopus* hamster is a small rodent that differs taxonomically from the Syrian hamster, which is the major laboratory hamster. We recently determined that this hamster is a good candidate for a new laboratory animal and have established an inbred strain named PMI. A PMI hamster with a morphologically abnormal stomach was found on anatomical screening in May 2010. Pathological observation suggested this abnormality represented a well-differentiated adenocarcinoma. By successive anatomical screening of 41 PMI hamsters, we confirmed the occurrence of morphologically abnormal stomachs in 39 of 41 hamsters (95.12%) at a mean age of 309 days (range, 196 to 515 days). On the other hand, all 12 hamsters of the inbred TAK strain, which was established from *Phodopus campbelli* in 2009, showed normal stomachs at a mean age of 256 days (range, 236 to 293 days). The high incidence of morphologically abnormal stomachs was thought to be a special feature of the PMI inbred strain.

Filarial nematode diverts thermoregulation to developmental transition following transmission by Aedes mosquito

Parasitic nematodes of humans, livestock, and other animals cause diseases of major socioeconomic significance globally. The parasitic nematode employing obligate diapause arrests development at this particular stage in each generation. Recovery to the progressive stage from diapause is induced upon host infection by both intrinsic and extrinsic (environmental) elements of the host. *Dirofilaria immitis*, a filarial nematode, develops through a series of 4 molts during the transmission cycle between mammalian hosts and mosquito vectors. The L1 and L2 larvae mature in the mosquito's body before reaching the infective larval stage (L3), and further developmental transition (re-initiation) occurs just after transmission to the host. We found that the combination of proper temperature (37°C) and nutrition supply is required for the filarial L3 to resume development with acute expression of stress marker *hsp70*, which was rapidly decreased in *D.*

immitis but showed sustained expression in *Caenorhabditis elegans*. Unordinary duplication of a part of the kinase domain ensured the poor activation of the stress kinase c-Jun N-terminal kinase in response to 37°C. Both *cuticlin-1* and *cathepsin-L*, identified as thermoregulated genes, were required for adequate L3 molting. Taken together, our findings suggest that thermoregulation for adapting to environmental change also exerts control over the developmental transition in filarial parasites.

Radioisotope Research Facility

Kunihiko Fukuda, *Professor and Director*

Yukio Yoshizawa, *Assistant Professor*

General Summary

The Radioisotope Research Facility was established to support medical and biological research with radioisotopes. The Facility also accepts non-radioisotopic research. We have supported researchers by suggesting methods and practical techniques for experiments. Lectures and training courses are held for researchers and for medical students and graduate students. In 2012, 35 researchers from 12 departments and 13 students of 2 curriculums used the laboratory of this facility. Major nuclides used for experiments were ^{32}P , ^{51}Cr , ^{125}I , ^{35}S , and ^3H .

The Fukushima Dai-ichi Nuclear Power Plant was damaged by the Tohoku-Pacific Ocean Earthquake and Tsunami on March 11, 2011. Large amounts of fission products and activation products were released into the environment by the accident. We focus on the study of the behavior and distribution of the radioactive materials in the environment as well as on radiation biology. Education related to radiation is also an interest.

Research Activities

Radioactive fallout in the environment

Radioactive materials from the accident at the Fukushima Dai-ichi Nuclear Power Plant spread as far as the Kanto area. Soil and plant samples were examined with radiation images using an imaging plate system.

Imaging data of a bamboo shoot in Kawamata-machi, Fukushima Prefecture, from June 2011 suggest that its contamination by ^{134}Cs and ^{137}Cs was caused not by extraneous attachment but by absorption through roots or the translocation from leaves. Radioactive cesium uptake into the plant body was present in high concentrations in the edible parts of the bamboo shoot, especially at the tip. We will continue to collect imaging data in the same bamboo grove to obtain more information on annual trends and the circulation of radioactive cesium in the bamboo forest.

Radiation images of leaves show small particles with high radioactivity. These radioactive particles had moved little from the initial site of adhesion despite repeated exposure to rain. Particulate contamination was distributed only in the bottom leaves that had opened before the accident and was not seen in the top leaves that grew after the accident. This finding confirms that particulate deposition on the leaves and the bark would remain for several years and that concentrations of radiocesium in whole leaves would decrease as new leaves grow.

Analysis of resistance mechanism in radiation resistant organisms

Tardigrades are small 8-legged animals less than 1 mm in length. They are commonly called water bears because of the way they walk slowly in water like bears. Water bears

are tolerant to ionizing radiation. They inhabit environments ranging from the deep sea to the land. Activated sludge was obtained from the Ariake Water Reclamation Center, Bureau of Sewerage, Tokyo Metropolitan Government, to clarify the radiation-resistant mechanism of water bears in the sludge. DNA-based identification of the water bears in the activated sludge revealed that only those of the genus *Isohypsibius* were present.

Isohypsibius was exposed to ionizing radiation using the cobalt-60 irradiation equipment at Takasaki Advanced Radiation Research Institute, Japan Atomic Energy Agency. The absorbed dose range for the irradiation was 5, 2, 1, and 0.5 kGy with 0 kGy as a negative control. The results show that *Isohypsibius* was tolerant to the dose of 2 kGy but that a dose of 5 kGy was fatal. A fluorescence dye, CellTracker Green CMFDA (Lonza Walkersville, Inc., Walkersville, MD, USA), affects the radiosensitivity of water bears. Therefore, CellTracker was added at concentration of 1 μM to the culture solution of *Isohypsibius*, which was then irradiated with doses of 375, 250, and 125 Gy using an X-radiation device (MBR-1520R, Hitachi Medico, Tokyo, Japan). The presence of CellTracker increased the radiosensitivity of *Isohypsibius* such that the dose of 250 Gy was fatal.

Study of products containing radioactive materials of natural origin

We have developed a silicone oil scintillator to measure radon in air and water. Radon is a chemically inert, naturally occurring radioactive gas that emanates from rocks and soils. The World Health Organization has proposed a reference level of 100 Bq/m³ in 2009 for the purpose of controlling lung cancer caused by radon gas. Under the prevailing country-specific conditions, the chosen reference level should not exceed 300 Bq/m³, which represents a biological dose equivalent of approximately 10 mSv per year. Uranium and thorium concentrations in the soil are relatively low in Japan, and the indoor radon density in most houses is less than 100 Bq/m³. However, there are bath additives that contain radioactive materials marketed as so-called “radon hot spring.” These radioactive materials of natural origin are regulated by “guidelines for the safety management of naturally occurring radioactive materials including uranium or thorium.” Therefore, we attempted qualitative and quantitative analyses of the “thoron hot spring” manufactured with a radioactive hot spring-making device using amang (tin tailing) that contains thorium. The peaks of ²³²Th and ²²⁴Ra were confirmed by the measurement of alpha-rays using a liquid scintillation counter (LSC-6100, Aloka, Tokyo, Japan). On the other hand, the presence of ²¹²Pb (239 keV) and ²²⁸Ac (338 keV) was confirmed by the measurement of gamma-rays. The radioactivity of these substances was lower enough than 1 Bq/g, which is the upper limit for regulation in the guidelines.

Core Research Facilities

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

Hiroyuki Sasaki, *Associate Professor*

General Summary

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

1. Annual Registration System

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

2. System for Providing Research Services

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

Research Activities

Monoclonal antibody for diagnosis of thyroid papillary carcinoma

Previously a monoclonal antibody recognizing thyroid carcinoma was established in the University by immunizing the membrane fraction of tumor cells from a patient with papillary thyroid carcinoma. This antibody recognizes an antigen produced by malignant thyroid carcinoma cells. By conjugating the antibody, we have attempted to develop an assay system from the blood of patients. The antibody was conjugated to streptavidin, and a method of sandwich enzyme-linked immunosorbent assay was devised. This

method enabled us to perform highly sensitive detection and quantification of the antigen. To test the relevancy of this assay, a clinical trial study is being performed.

Analysis of the responsive gene of spontaneous mutant ICR Kuru² mouse

We have established a mouse model of spontaneous deafness by sibling-inbreeding over 10 years. The mouse was designated as kuru² and is kept in the University. To identify the genetic abnormality, the mouse was back-crossed to *Mus musculus castaneus* (CAST), and myosine 15 or myoXV on chromosome 11 was assumed to be the responsive gene. This year, the background abnormality was identified with gene sequencing. A deletion of 2,446 base pairs was found in the mouse (from 28,795 to 31,241 in the complete sequence of the *M. musculus* unconventional myosin-15 gene; National Center for Biotechnology Information accession: AF144093). The myosin ATP-binding site is present in the deleted area. Considering the function that the affected area regulates and previous reports, hearing loss of the examined mouse is attributable to the abnormality of the myoXV gene, and we reported that the mouse might be another type of shaker-2 deaf mouse.

Peptide nanovesicles formed by the self-assembly of branched amphiphilic peptides

Peptide-based packaging systems show great potential as safer drug-delivery systems. They overcome problems associated with lipid-based or viral delivery systems in terms of stability, specificity, inflammation, antigenicity, and tuneability. Here, we describe a set of 15- and 23-residue branched, amphiphilic peptides that mimic phosphoglycerides in molecular architecture. These peptides undergo supramolecular self-assembly and form solvent-filled, bilayer-delimited spheres with 50.200-nm diameters as confirmed with transmission electron microscopy, scanning transmission electron microscopy, and dynamic light scattering. Whereas weak hydrophobic forces drive and sustain lipid bilayer assemblies, these all-peptide structures are stabilized potentially by both hydrophobic interactions and hydrogen bonds and remain intact at low micromolar concentrations and higher temperatures. A linear peptide lacking the branch point showed no self-assembly properties. We have observed that these peptide vesicles can trap fluorescent dye molecules within their interior and are taken up by N/N 1003A rabbit lens epithelial cells grown in culture. These assemblies are thus potential drug-delivery systems that can overcome some of the key limitations of the current packaging systems.

Publications

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Department of Genetic Diseases and Genome Science

Yoshikatsu Eto, *Professor*

Torayuki Okuyama, *Visiting Professor*

General Summary

The main research topics in the Department of Genetics and Genome Science are the basic pathogenesis of genetic diseases, particularly, lysosomal storage diseases (LSDs), and the development of therapies for LSDs. Of our research topics, the pathogenesis of central nervous system (CNS) involvement in LSDs is the most important. To understand the pathophysiology of CNS events in LSDs, we generated induced pluripotent stem (iPS) cells from mucopolysaccharidosis (MPS) VII mice and caused them to differentiate into neuronal cells. We also generated iPS cells from a mouse model of Pompe disease and caused them to differentiate into skeletal muscle cells. We can produce disease models of various LSDs using iPS technology. Recently, we generated iPS cells from human patients with Gaucher, Fabry disease, and Pompe disease. Furthermore, we treated CNS involvement of LSDs by means of intrathecal injection of enzymes into MPS II mice. Intrathecal injection of enzymes was extremely effective for treating the CNS involvement of lysosomal diseases. These findings indicate that intrathecal treatment is feasible for treating the CNS in various LSDs.

Research Activities

1. iPS cells from various LSDs are important research tools for understanding the pathophysiology of LSDs and can also be applied to the treatment of LSDs. We successfully generated iPS cells from Pompe mice by means of tail-tip fibroblasts, mouse embryonic fibroblasts, and 3 factors: Klf4, Sox2, and Oc2/4t. The iPS cells differentiated into skeletal muscle cells. Pompe skeletal muscle cells showed massive accumulation of glycogen in lysosomes surrounded by a single membrane unit. We also generated iPS cells from patients with Gaucher disease, Fabry disease, and Pompe disease.

2. Development of treatment procedures for LSDs

Establishing novel treatment procedures for the CNS involvement of LSDs is our most important project. One procedure is intrathecal or intraparenchymal injection of enzymes into MPS II mice. We found that intrathecal injection produced significant elevations of enzyme activities in various regions of the brain and in other organs, such as the liver, spleen, kidney, and heart. Furthermore, histological improvement in the brains of MPS II mice was also observed.

3. The screening for LSDs with dried blood spots is an important technology for the early diagnosis and treatment of patients with Fabry, Pompe, and MPS I, II, IV, and VI. We used the fluorometric assay method to establish the dried blood spot method for the early diagnosis of Pompe disease, Fabry disease, Morquio syndrome (MPS IV), and MPS VI. Furthermore, we recently established a dried blood spot diagnostic method for Wolman disease.

4. Development of gene and cell therapy: Basic research using lentiviral vectors or adeno-associated vectors in a mouse model of MPS II was performed to develop gene and cell therapies for human LSDs, including Pompe disease, Fabry disease, and MPS VII.

5. Pathological analysis and generation of iPS cells from LSDs

We revealed the ultrafine structural characteristics of iPS cells from human Pompe disease, Fabry disease, and MPS.

6. Educational activities for patients with LSDs

We held educational seminars for patients with Fabry disease 3 times this year and plan to continue doing so in the future.

7. We organized seminars about genetic diseases (including LSDs) for medical students 3 times this year and held a public seminar in the Tokyo area.

8. International symposium of gene therapy

This symposium, held on January 17, 2012, was attended by about 120 persons. The aim of the meeting was to encourage international cooperation for the establishment of a gene therapy system for genetic diseases.

9. Educational activities about LSDs

To increase recognition of LSDs by physicians, medical students, and the general public, we are creating a pamphlet, holding patient seminars, organizing research meetings, and creating websites.

These activities are very important in the future

In the study of LSDs, research on iPS cells, screening methods, and educational programs have achieved much, internationally and in Japan, and have led to beneficial social, scientific, and academic outcomes.

Inspection and evaluation

Screening with blood spot methods have contributed to the early diagnosis of patients at high-risk for LSDs in Japan. In collaboration with the Department of Gene Therapy, Institute of DNA Medicine, we accomplished several of these topics. In the study of LSDs, research on iPS cells, screening methods, and educational programs have achieved much, internationally and in Japan, and have led to beneficial social, scientific, and academic outcomes.

Research achievements

Intrathecal administration of enzymes could be very useful for treating CNS manifestations of LSDs, such as MPS II. Furthermore, iPS technology will contribute to our understanding of the pathogenetic mechanism of LSDs.

Publications

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CD3 antibodies modulates the immune response to an infusion of α -glucosidase in mice. *Mol Ther.* 2012; **20**: 1924-31.

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Department of Allergology

Naohiro Watanabe, *Professor and Director*

Hirohisa Saito, *Professor*

General Summary

The department of allergology was established last year. Our research concerns the biological significance of allergic immune reactions by immunoglobulin E (IgE), mast cells, eosinophils, and basophils.

Research Activities

Mast cells in atopic dermatitis

We first reported on the NC/Nga mouse as a model of atopic dermatitis in which skin lesions develop spontaneously through a genetic predisposition and a continuing exposure to environmental antigens. Our previous experiments indicated that mast cells and a chymase derived from them are involved in the development of atopic dermatitis in NC/Nga mice. We have found a novel inhibitor of chymase which suppresses the development of atopic dermatitis. We examined the dosages of oral administration of the inhibitor to decrease the severity of atopic dermatitis in NC/Nga mice. The relationship between plasma levels of the inhibitor and clinical skin score showed that the minimum effective plasma concentration of this inhibitor was 0.13 to 0.2 ng/ml and that duration times of over the effective dosages were 10 to 12 hours. These findings predicted a dosage for treating atopic dermatitis in human patients. A clinical trial of this inhibitor is under way. The inhibition of mast cell-specific chymase is a new idea in the therapy of atopic dermatitis.

Protection against parasites by innate immunity

Myeloid differentiation protein (MD)-1 has been found as an analogue of MD-2 and is considered to be a factor of innate immunity. MD-1 is expressed on antigen-presenting cells and on cells in the lung, spleen, and kidney. To clarify the function of MD-1, experiments were performed which compared the protective immunity against intestinal nematodes between MD-1 $-/-$ and wild-type mice. The number of muscle larvae after *Trichinella spiralis* infection in MD-1 $-/-$ mice was half that in wild-type mice. Egg output after *Nippostrongylus brasiliensis* infection was less in MD-1 $-/-$ mice than in wild-type mice. The numbers of eggs and adult worms after primary and secondary infection with *Heligmosomoides polygyrus* were significantly less in MD-1 $-/-$ mice than in wild-type mice. These results indicate that MD-1 has a suppressive effect on protection against nematodes and suggest that MD-1 is a negative element of innate immunity. The protective immunity against these nematodes is believed to depend on a helper T type 2 (Th2) response. Therefore, MD-1 as a factor of the innate immune system is likely to interact with the Th2 acquired immune system.

Publications

Furuta T¹, Murao LA², Lan NT³, Huy NT², Huong VT³, Thuy TT⁴, Tham VD⁵, Nga CT⁶, Ha TT², Ohmoto Y⁶, Kikuchi M², Morita K², Yasunami M², Hirayama K², Watanabe N (¹Univ Tokyo, ²Nagasaki Univ, ³Pastuer Inst, ⁴Children Hosp Viet Nam, ⁵Cent Prev Med Viet Nam, ⁶Otsuka Co., LTD). Association of mast cell-derived VEGF and proteases in Dengue shock syndrome. *PLoS Negl Trop Dis*. 2012; **6**: e1505.

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Watanabe N. Trypanosomiasis (in Japanese). In: Nagai R, Ohta K, editors. *Konnichi no chiryou to kango*. 3rd ed. Tokyo: Nankodo; 2013. p. 963.

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Department of Molecular Physiology

Division of Physical Fitness

Shigeru Takemori, *Professor and Director*

Hideki Yamauchi, *Assistant Professor*

General Summary

Research activities in our division have been focused on the plasticity of skeletal muscle and preventive medicine against metabolic syndrome in terms of exercise physiology.

Research Activities

Myostatin physiologically regulates skeletal muscle metabolism with growth and exercise through the adenosine monophosphate kinase signaling pathway

Recent studies of the myostatin knockout mouse have suggested that myostatin, a factor suppressing the growth of skeletal muscle, regulates carbohydrate and lipid metabolism in skeletal muscles. To validate this notion under physiological conditions, the effects of postnatal up-regulation of myostatin in combination with the effects of exercise were examined with a focus on the involvement of the adenosine monophosphate kinase (AMPK) signaling pathway. We found that myostatin expression was increased with growth (from 6 weeks to 14 weeks) independently of exercise loading. Also, the expressions of phosphorylated AMPK α and peroxisome proliferative activated receptor- γ co-activator 1 α (PGC-1 α) protein decreased correspondingly, with concomitant down-regulation of carbohydrate and lipid metabolism. These decreases were inhibited by voluntary running exercise. We confirmed that myostatin physiologically regulates carbohydrate and lipid metabolism in skeletal muscle cells via the AMPK-PGC signaling pathway.

Exercise training-induced improvement in impaired muscle glucose metabolism is associated with muscular sex-steroid hormone level in Zucker fatty rats

The effect of exercise training in patients with type 2 diabetes to decrease hyperglycemia via enhancement of muscle glucose metabolism signaling is well known. We have recently reported that sex steroid hormones can be synthesized locally in skeletal muscle and can decrease fasting blood glucose levels in obese rats. We further examined whether exercise training-induced secretion of muscular sex-steroid hormones decreases insulin resistance in Zucker fatty rats. We found that 6 weeks of exercise training decreased serum insulin and fasting glucose levels. Muscle concentrations of dehydroepiandrosterone and dihydrotestosterone and protein expression levels of 5 α -reductase increased with exercise training. In addition, exercise training upregulated translocation of glucose transporter type 4 with concomitant increases in protein kinase B and protein kinase C- ζ/λ phosphorylation. Furthermore, significant correlations were observed between fasting glucose levels and muscular levels of dehydroepiandrosterone and dihydrotestosterone. Interestingly, exercise training-induced improvements in serum insulin

and fasting glucose levels concomitant with glucose transporter type 4–regulation signals were all suppressed with a 5 α -reductase inhibitor. These results indicate that exercise-induced improvements in muscle glucose metabolism signaling and fasting glucose levels may be attributed to the increased muscular levels of sex steroid hormones.

Effects of habitual exercise and diet restriction on fatty liver in Zucker fatty rats

Diet restriction and physical exercise have been used for the prevention and treatment of obesity. We have found that voluntary exercise with diet restriction induced improvements in the fatty pancreas of Zucker fatty rats, but diet restriction alone did not. Here, we investigated whether voluntary exercise or diet restriction of both improves fatty liver in Zucker fatty rats. Voluntary exercise and diet restriction decreased obesity and insulin resistance. Hepatic fat accumulation was facilitated by diet restriction alone and was suppressed by voluntary exercise with diet restriction via a decrease in fatty acid synthase activity. The results indicate that exercise training combined with diet restriction, but not diet restriction alone, is important to prevent fatty liver. In addition, recent studies have been found that Toll-like receptors (TLRs) play an important role in the links among insulin resistance, inflammation, obesity, and type 2 diabetes. Thus, we examined messenger RNA expression of TLRs in the liver and found that voluntary exercise tended to inhibit the expression of TLR2 but not of TLR4. These results suggest that exercise training and diet restriction do not markedly affect messenger RNA expression of TLRs in the livers of Zucker fatty rats.

Publications

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mediated by DHT produced in skeletal muscle of Zucker diabetic fatty rats. *J Diabetes Metab.* 2013; **4**: 239.

Department of Cell Physiology Division of Aerospace Medicine

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

Masamichi Sudoh, *Professor*

General Summary

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

Research Activities

Gravitational physiology and aerospace medicine

1. Technique of electrocardiographic recording using medaka

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

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

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

2. Research on visual stimulus and posture control

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

3. Outreach activities for aerospace medicine

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

Physioepidemiological study of health

Many previous studies of wellness medicine and occupational health have been performed with epidemiological methods. However, we analyzed physiological data (e.g., ECG) and used epidemiological methods. We believe that mental stress and human health can be evaluated objectively with both physiological methods and epidemiological methods.

1. The effect of the musical ensemble on the human health: We evaluated the effect of a musical ensemble on autonomic nervous function through use of a respiratory function test and a circulatory function test. Respiratory and circulatory functions were observed to synchronize in the musicians of the ensemble. We believe that this result might lead to the development of a novel musical therapy.
2. Mental stress in care workers: Our questionnaire survey revealed that the stress of care workers was significantly higher than that of nurses. However, direct measurements with Holter ECG monitoring as they performed their duties revealed no significant difference in stress between care workers and nurses. We speculate that care workers are less able to deal with stress because they have had few opportunities to learn about stress-management.

Department of Pathology

Division of Neuropathology

Hiroshi Hano, *Professor and Director*
Junko Fujigasaki, *Assistant Professor*

Takahiro Fukuda, *Assistant Professor*

General Summary

Our research projects have concerned neurodegenerative disorders caused by intracellular accumulation of abnormal proteins. We are also studying mouse models of neurodegenerative disorders and autopsy cases by means of standard morphologic analysis and molecular biological analysis.

Research Activities

Pathophysiological study of neuronal organelles in lysosomal disorders

Objective: This study investigated the pathophysiology of neuronal organelles in lysosomal disorders.

Material and methods: We analyzed the central nervous system of mouse models of Niemann-Pick disease type C and prosaposin deficiency with immunohistochemical techniques and antibodies against early endosome antigen 1, trans-Golgi network protein 38 (Golgi apparatus), cytochrome c oxidase subunit IV (mitochondria), calnexin (endoplasmic reticulum), S6 ribosomal protein, lysosomal-associated membrane antigen 2, and catalase (peroxisome).

Results: In the central nervous system neurons of Niemann-Pick disease type C and prosaposin-deficiency mice, swollen lysosomes accumulated. Structurally preserved peroxisomes and Golgi apparatuses decreased slightly in number. Mitochondria, endosomes, endoplasmic reticulum, and ribosomes decreased markedly in number.

An autopsy case of frontotemporal lobar degeneration with motor neuron disease (primary lateral sclerosis type)

Flaccid dysarthria developed in a 68-year-old man. At age 69 years, he acted in a conspicuously selfish manner and exhibited forced laughter with mild dementia (Hasegawa Dementia Scale, Revised: 22 of 30). He showed upper motor neuron signs, including hyperreflexia, spasticity, Babinski sign, and paralysis. He died of pneumonia at 71 years of age. A postmortem examination of the brain, which weighed 1,150 g, revealed degeneration of the corticospinal tract with complete loss of Betz cells. The affected frontal and temporal cortices showed diffuse neuronal loss, with microvacuolar change and phosphorylated 43-kDa transactivation response DNA-binding protein (pTDP43)-positive neuronal cytoplasmic inclusions and pTDP43-positive dystrophic neurites in all cortical layers. No pTDP43-positive neuronal intranuclear inclusions could be detected. There was no obvious lower motor neuron loss or Bunina bodies in the hypoglossal nucleus or spinal cord, whereas the fragmentation of Golgi apparatuses was observed in lower motor

neurons with pTDP43-positive inclusions.

An autopsy case of Chagas disease

An 80-year-old Japanese–Brazilian man had lived in urban areas of Brazil and moved to Japan at 62 years of age. He was admitted with a complaint of somnolence. Diffusion-weighted magnetic resonance imaging showed multiple areas of high signal intensity in the deep white matter. Physical examination revealed megacolon. *Trypanosoma cruzi* organisms were identified in the blood. He died of chronic Chagas disease. A postmortem examination of the brain, weighing 1,320 g, revealed infiltration of macrophages, lymphocytes, and plasmacytes into perivascular areas and *T. cruzi* organisms in intravascular and perivascular areas and in the cytoplasm of astrocytes, macrophages, and neurons. Neuronal loss and ischemic cerebral changes and demyelination were also observed. Chagas disease has a long incubation period and is often refractory to treatment. Awareness that Chagas disease is now found in places far from areas of Latin America where it is endemic is important because it leads to the development of strategies to prevent potential sources of transmission.

Nuclear inclusions and Cajal bodies in spinocerebellar ataxia 7

Spinocerebellar ataxia 7 (SCA7) is an autosomal dominant neurodegenerative disorder characterized by cerebellar ataxia and retinal degeneration. SCA7 is caused by a polyglutamine expansion in ataxin-7. The pathologic hallmark of SCA7 is the formation of neuronal intranuclear inclusions (NIIs) and accumulation of mutated ataxin-7. Nuclear functional domains related to the formation of NIIs, especially promyelocytic leukemia nuclear bodies, could be accumulation sites of the pathological ataxin-7 with expanded polyglutamine. Cajal bodies, which have been implicated in RNA-related metabolic processes, might be related to the formation of NIIs in SCA7. In this study, we examined the relation of Cajal bodies and NIIs in the brains humans and mice with SCA7. Coilin, a molecular marker of Cajal bodies, were attached to or entrapped in ataxin-7-positive NIIs in the human and mouse brains. The protein survival of motor neurons, a marker of Gemini of Cajal bodies, is also found in the NIIs. These findings indicate that alteration of RNA metabolism might be related to the pathogenesis of SCA7.

Publications

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Department of Orthopaedic Surgery

Division of Sports Medicine

Keishi Marumo, *Professor*

Hiroki Funasaki, *Associate Professor*

General Summary

Clinical Research

The ongoing research in our department concentrates on competitive athletes (including professionals), amateurs who include sports activities in their daily lives, and young athletes practicing sports in school sports clubs or dedicated to training within sports clubs. This outline focuses on the basic research performed in 2012.

Research Activities

Comparison of muscle potential silent periods between operated and nonoperated sides in patients after reconstruction of the anterior cruciate ligament

The purpose of this study was to compare motor unit functional variables — premotion time, premotion silent period, and switching silent period — between operated and non-operated knees in patients following reconstruction of the anterior cruciate ligament. Six patients were enrolled. They were examined at postoperative follow-up, 8 to 12 months after the surgery. Electromyographic data of the rectus femoris and biceps femoris muscles were recorded during jumping: the patients were asked to jump on one leg as quickly as they could in response to a flashing light. Although there was no significant difference in premotion time between the operated and the nonoperated sides, the premotion silent period and the switching silent period were significantly longer on the operated side than on the nonoperated side. The results suggest that the nerve-muscle coordination on the operated side deteriorates 8 to 12 months after reconstruction of the anterior cruciate ligament. The silent period has been suggested to be a useful variable for assessing motor unit function in athletes undergoing rehabilitation and has been proposed as a tool for guiding the return to sports activity.

Three-dimensional gait analysis in patients with bilateral knee osteoarthritis before and after total knee arthroplasty

The purpose of this study was to compare the data of 3-dimensional gait analysis with a motion analysis system (Vicon Motion Systems, Ltd., Oxford, UK) in 12 patients with bilateral knee osteoarthritis who had undergone bilateral total knee arthroplasty.

Analyzed variables were: 1) step length, 2) walking speed, 3) percentage of single-leg support phase, 4) ground force during the single-leg support phase, 5) step width, and 6) range of motion of the hip, knee, and ankle joints. Step length, walking speed, percentage of single-leg support phase, ground force during the single-leg support phase, and range of motion improved significantly in patients who had preoperative Japanese Orthopaedic Association scores of 60 points or more. On the other hand, in patients with

scores of less than 60 points, significant improvements were not observed. We conclude that various gait variables improve after total knee arthroplasty, although patients with severe osteoarthritis (Japanese Orthopaedic Association score of less than 60 points) showed less improvement in walking ability.

The results of Bankart repair and the usefulness of a rehabilitation program we designed for the return of students to sports

We studied clinical characteristics and results of Bankart repair in 36 patients (students engaged in some sports activity) with recurrent dislocation of the shoulder and evaluated the usefulness of a rehabilitation program we developed for their return to sports after surgery. In most patients, the initial dislocation had occurred during sports activity. Patients underwent surgical treatment after dislocation had recurred 3 or 4 times. Many patients continued their sports activity after surgery, and their return to sports was facilitated by introduction of our rehabilitation program.

Pain of the anterior superior iliac spine in junior-high and high school soccer players

We reported on 3 patients who had pain in the anterior superior iliac spine (ASIS) area while playing soccer. Magnetic resonance showed high signal intensity in the bone marrow, apophysis, epiphyseal line, and the attaching muscles. We speculated that these high signal intensities were caused by chronic stress in the muscles attaching to the ASIS and that lesions might act as triggers leading to ASIS avulsion fracture.

Publications

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Reviews and Books

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Health-Care Center

Mikio Zeniya, *Professor and Director*
 Takashi Wada, *Professor*
 Hiroki Takahashi, *Assistant Professor*
 Hiroko Nogi, *Assistant Professor*

Yoichi Sakamoto, *Professor*
 Takekazu Onda, *Professor*
 Tsutomu Fukumoto, *Assistant Professor*
 Yuhshi Kuniyasu, *Assistant Professor*

General Summary

The most outstanding activity during the 2012-13 year was the organization of the annual meeting of the Japanese Society of Ningen Dock 2012. Many studies using the database, which was provided by the Shimbashi Medical Checkup Office (a section of the Health-Care Center), were presented or published.

Our study of the potential utility of perimetry to identify patients with glaucoma was selected to receive a researcher's award from the *Nihon Sogo Kenshin Igakukai*.

Research Activities

A relationship between the incidence of metabolic syndrome and the frequency of feeding
 Healthy persons (3,949 men and 2,155 women) aged 30 to 59 years were selected and divided into groups based on how often they ate breakfast. After 2 years the incidence of metabolic syndrome was compared between the groups. The incidence of metabolic syndrome was significantly lower for groups that ate breakfast 0 to 1 times a week or 6 to 7 times a week than for other groups (7.59% vs. 11.13%).

A study of serum alkaline phosphatase variability

Alkaline phosphatase (ALP) is a common and well-established indicator of hepatic and biliary disorders, although it varies under many conditions. We focused on age, sex, blood type (A/B/O), and menstrual status to define the magnitude of serum ALP level variability. We found that female subjects showed age-dependent increases in serum ALP levels and that the increases correlated with the time since menopause. An evaluation of blood type showed that serum ALP levels were higher in subjects with type B or O. The greatest difference was between type O (218 ± 49 U/L) and type AB (186 ± 44 U/L) in male subjects. Because of these results, a new "normal" range for serum ALP is under discussion.

Respiratory function and smoking, body weight

From the standpoint of preventive medicine, preventing people from smoking is an important task. To educate the general public, "lung age," a variable calculated from height and forced expiratory volume in 1 second, is persuasive. According to our database, current smokers have a "lung age" 9.8 ± 14.7 years greater than their chronological age, whereas that of past smokers is 4.8 ± 17.8 years greater, and that of nonsmokers is 0.8 ± 14.7 years less. Moreover, a comparison of weight loss and "lung age" showed that weight loss is proportional to the decrease in "lung age."

The potential utility of perimetry at physical examinations to identify persons with glaucoma

Recent studies show that approximately 90% of cases of glaucoma in Japan are normal-pressure glaucoma, which cannot be detected with screening ophthalmotonometry. We used perimetry at physical examinations to identify persons suspected of having glaucoma; of these persons, 45 who were finally found to have as glaucoma underwent ophthalmotonometry. All 45 subjects had normal intraocular pressure, which indicates the potential utility of perimetry at physical examination for identifying persons with glaucoma.

The study was selected to receive a researcher's award from the *Nihon Sogo Kenshin Igakukai*.

Publications

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Reviews and Books

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Premedical Course

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

The mechanism of engulfment of the generative cell by the pollen tube cell

During the ontogeny of angiospermous pollen, a small generative cell (GC) is produced by unequal cell division of a microspore. The GC subsequently detaches from the pollen grain wall (intine) and is engulfed within the cytoplasm of the pollen tube cell (PC) to create a “cell within a cell” structure. Then the GC is allowed to migrate through the pollen tube. Although intracellular migration of the GC is required for successful fertilization, the manner of detachment of the GC is poorly known. In this study, the GC detachment mechanism, especially the roles of cytoskeletal elements, were investigated in *Liriope muscari* pollen. This species produces lenticular GC on the side of the pollen grain through microspore division. Soon after the division, methyl-esterified pectins and callose were deposited in the cell wall between the GC and the PC. However, they subsequently disappeared, and the cell wall became thin. The GC underwent circumferential constriction near its attachment site on the intine, it thereby became spherical and projected into the PC. The constriction finally became so tight that opposing ends of the cell membranes met and fused. The GC detached from the intine to be engulfed within the PC. Actin filaments were localized around the GC, particularly the constriction domain. Cytochalasin B, an actin inhibitor, and 2,3-butanedione monoxime, an ATPase inhibitor of myosin, prevented the GC from being engulfed within the PC. On the other hand, colchicine, a microtubule inhibitor, did not affect GC engulfment. These results suggest that the softening of the cell wall through the degradation of pectins and callose and the constriction by actin-myosin interaction play important roles in the engulfment process of the GC.

Publications

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Physics

Tsuyoshi Ueta, *Professor*

Katsumi Kasono, *Assistant Professor*

General Summary

1. Since 1998, by artificially introducing lattice vibration to a photonic crystal, we have investigated the direct interaction of incident light and lattice vibration and have found out that the incident light is amplified. We have proposed a metal photonic crystal as a system enhancing the dynamic Casimir effect, and have been investigating the properties of the dynamic Casimir effect within a metal photonic crystal.
2. Phase transitions, critical phenomena, interacting many-body systems, computer simulation.

Research Activities

New superconducting phase in a high magnetic field

The microscopic theory of superconductivity in a magnetic field has been studied by reforming the Gor'kov theory in terms of the exact Green function in a magnetic field. We have found that a superconducting domain exists on the higher-field side of the domain according to the parabolic law. These results were presented at the International Conference on Superconductivity and Magnetism 2012 held in Istanbul, Turkey.

Monte Carlo simulations of the $q=10$ Potts models

We have performed Monte Carlo simulations to study systems with the first-order phase transition. Multigrid method Cluster Monte Carlo simulations are used to study 10 state ferromagnetic Potts models on square lattices. We calculated the relaxation times of energy and magnetization.

Publications

Ueta T, Miyagawa Y (Chiba Univ). Local gauge finite element method for electron waves in magnetic fields. *Phys Rev E Stat Phys Plasmas Fluids Relat Interdiscip Topics*. 2012; **86**: 026707.

Ueta T, Hioki T (Chiba Univ). Gor'kov theory with exact green function in magnetic field. *Journal of Superconductivity and Novel Magnetism*. 2013; **26**: 1921-6. Epub Dec 22, 2012.

Fujii G (Akita Pref Univ), Ueta T. Finite element analyses for random laser action in metallic disordered structures. In: *Metamaterials 2012: 6th International Congress on Advanced Electromagnetic Materials in Microwaves and Optics Proceedings*; 2012 Sep 17-22; St. Petersburg, Russia. *Metamorphose VI*; 2012. p. 758-60.

Chemistry

Takashi Okano, *Professor*

Chikao Hashimoto, *Associate Professor*

General Summary

The research of this laboratory is focused on synthesis-oriented organic chemistry, including the synthesis of bioactive compounds and fluorine-containing materials; the development of new methods for peptide synthesis; and the computer-assisted analysis of materials and synthetic reactions.

Research Activities

Synthesis of a new facile diagnostic agent for Fabry disease

In collaboration with the Department of Gene Therapy, Institute of DNA Medicine, we are developing a new fluorescent diagnostic agent for Fabry disease, a congenital deficiency of α -galactosidase. The difficulty of the synthesis is to create a precise α -glycoside linkage with the fluorescein-based fluorescent moiety and galactose to avoid the effect of β -galactosidase. In addition, we are developing a more readily available ^{13}C -labeled agent that can be detected with mass spectrometry as a new diagnostic indicator.

*Synthesis of *N*-protected peptide acids using amino acid-alkaline earth metal salts*

The protection of a carboxyl group by a metal ion saves the time needed for the incorporation and removal of the protecting group and prevents side reactions caused by the use of esters. The syntheses of *N*-protected peptide acids in organic solvents using alkaline earth metal-carboxylate salts of an amino acid were investigated. We found that the amino acid-Ca carboxylate salt is the most effective among the carboxylate salts of the amino acids tested for coupling with Boc-amino acid active esters in an organic solvent, such as *N,N*-dimethylformamide or dimethylsulfoxide.

Publications

Hashimoto C, Takeguchi K¹, Kodomari M¹
(¹*Shibaura Inst Technol*). An efficient synthetic method of *N*-protected dipeptide acids using

amino acid-calcium carboxylates in an organic solvent. *Synlett*. 2011; **2011**(10): 1427-30.

Social Science (Law)

Ryuichi Ozawa, *Professor*

General Summary

Problems of Constitutional Law in present-day Japan

Decision of the Constitutional Council (*Conseil Constitutionnel*) of France

Research Activities

Ozawa published the following articles and books from research activities in 2012.

Publications

Ozawa R. Crises of democracy in the state and the province (in Japanese). *Keizai*. 2012; 203: 41-51.

Reviews and Books

Ozawa R. Civil liberty of public servants and democracy (in Japanese). In: Sugihara Y, Higuchi Y, Mori H, editors. *Sengohougaku to kenpou-rekishi genjou tenbou*. Tokyo: Nihon Hyoronsha; 2012. p. 348-60.

Ozawa R. Procedure for revision of Constitution

(in Japanese). In: Kitano Hirohisa sensei tsuito-ronshu kankoiinkai, editor. *Nozeisha kenron no kadai*. Tokyo: Keiso Shobo; 2012. p. 135-58.

Ozawa R. Close-Up Kenpo (in Japanese). 2nd ed. Kyoto: Horitsubunkasha; 2012.

Ozawa R. 44: Rationalized parliament and budget power, 52: Part determinant of financial autonomy, 53: Financial autonomy (in Japanese). In: Tsujimura M, editor. *Furansu no kenpohan-rei*. Tokyo: Shinzansha; 2013. p. 216-9, 257-60, 261-3.

Human Science

Takao Fukuyama, *Professor*

General Summary

The Study of Western philosophy and ethics

Research Activities

A study of first year experience

I designed a workshop for the Conference of 37th Faculty Development held on December 1, 2012, at the Kokuryou campus premedical department. This Faculty Development Workshop was titled “The Workshop on First-Year Experience.” During the workshop I reported on the birthplace of first year experience, global current of trends, emplacement in the course of undergraduate education, type-classification of the curriculum, and a present situation and problems of The Jikei University. And I insisted that The Kokuryo

Department achieve its own total direction and posture over each aims of subunits.

Japanese

Ikuko Noro, *Professor*

General Summary

1. To develop materials for nursing students to study Japanese language
2. To study patients' decision-making

Research Activities

To develop materials for nursing students to study Japanese language

We developed a textbook and exercises for nursing students to study “study skills,” (hon- orifics, proverbs, and report-writing) and “social skills” (greetings, showing empathy, and rapport-building).

To study patients' decision-making

We studied how patients with cancer make decisions about chemotherapy to reveal the associations among their decision-making process, their preferred decision-making model, their actual decision-making model, and their satisfaction with their decision.

Publications

Noro I, Muramoto T, Yamaoka A. A video investigation of the effects of physicians' verbal explanations and attitudes concerning informed

consent on patients (in Japanese). *Ninchi Shin-rigaku Kenkyu*. 2012; **10**: 81–93.

Mathematics

Katsuya Yokoi, *Professor*

Hiroshi Shiraishi, *Assistant Professor*

General Summary

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

Research Activities

- I. We studied omega-limit sets and (strong) chain recurrent sets on topological dynam-

ics.

II. We discussed a resampling procedure in the estimation of optimal portfolios when the financial returns are of the class of time-varying autoregressive conditional heteroskedasticity processes. We also discussed an algorithm to solve a multiperiod portfolio-selection problem based on a bootstrap method.

Publications

Shiraishi H, Ogata H¹, Amano T², Patilea V³, Veredas D⁴, Taniguchi M¹ (¹*Waseda Univ*, ²*Wakayama Univ*, ³*CREST*, ⁴*ECARES*). Optimal portfolios with end-of-period target. *Advances in Decision Sciences*. 2012; ID703465.

Shiraishi H. A simulation approach to statistical

estimation of multiperiod optimal portfolios. *Advances in Decision Sciences*. 2012; ID341476.

Shiraishi H. Resampling procedure in estimation of optimal portfolios for time-varying ARCH processes. *Scientiae Mathematicae Japonicae*. 2012; **75**: 105-17.

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 to investigate how to make useful digital images and XML files of fifteenth century manuscripts, especially of the *Stonor Letters*.

Fujii joined a project to compile English textbooks for high-school English classes: *English Communication I, II, and III*. Along with the textbooks, Fujii is working on exercise materials and teacher's manuals. In addition, he published a self-study vocabulary exercise book for the Test of English for International Communication.

Research Activities

Making use of the results of the study, Ohara continued his research on the graphemes of the letters of the Stonors.

Fujii analyzed and collected authentic English materials to meet the level and the needs of high-school textbooks based on current teaching methodologies, theories, and research findings on learning English as a foreign language. These materials were used to compile textbooks following the revised teaching guidelines set out by the Ministry of Education, Culture, Sports, Science and Technology. Officially approved by the Ministry, the first textbook in the series, *World Trek-English Communication I*, and its instructional

aids, *World Trek-English Communication I Teacher's Book* and *World Trek-English Communication I Teacher's Manual*, were published.

Reviews and Books

Mochizuki M¹, Aizawa K², Allum P³, Sasabe N⁴, Hayashi Y⁵, Fujii T, Miura S⁶ (*¹Reitaku Univ, ²Tokyo Denki Univ, ³Rikkyo Univ, ⁴Toritsu*

⁵Aoyama High, ⁶Souka High, ⁶Tsurubunka Univ). *World Trek English Communication I*. Tokyo: Kiriara Shoten; 2013.

First Foreign Languages

Katsumi Suzuki, *Associate Professor*

General Summary

German contemporary literature

Research Activities

I am working on the topic of “the modern German literature of nonnative writers in German-speaking areas,” especially the works of Ilija Trojanow, who was born in Bulgaria and now lives in Vienna. His novel *The Collector of Worlds* deals with the 3 different worlds of India, Arabia, and Africa. I had done research on his discourse about India and Africa and the cultural background of this discourse. I have already published the results. I continue researching his discourse about Arabia and studying Arabic culture.

School of Nursing

Basic Nursing

Mayumi Kikuchi, *Associate Professor*
Sumiko Satake, *Assistant Professor*

Chieko Hanyu, *Assistant Professor*

General Summary

Major study areas in basic nursing include: 1) education on physical assessment and supporting techniques, 2) supporting techniques in daily living, 3) the history of nursing, 4) supporting patients with progressive motor dysfunction, and 5) nursing diagnosis.

Research Activities

Mayumi Kikuchi: To review the methods of nursing practice for patients with progressive motor dysfunction, Kikuchi qualitatively described how nurses working in a ward for patients with muscular dystrophy should handle clinical situations.

Chieko Hanyu: Hanyu performed a questionnaire survey of teachers to understand the current status of education on physical assessment accompanying the revision of the 2009 curriculum.

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

Nursing Administration

Midori Nagano, *Professor*

General Summary

Two studies have been performed: “Effective pressure ulcer measures in the hospital” and “Quality indicators of home care nursing for older persons.”

Research Activities

Effective pressure ulcer measures in the hospital

To elucidate structural indicators and effective pressure-ulcer measures related to the sense of influence among wound, ostomy, and continence nurses (WOCNs) and the inci-

dence of pressure ulcers, a questionnaire survey on systems of pressure-ulcer measures was administered to the WOCNs in charge of pressure-ulcer measures and nurse administrators, who were their direct superiors, at 425 hospitals.

Regarding interprofessional collaboration, “occupational therapists/physical therapists perform assessment for high-risk patients as necessary” was related to both the sense of influence and the incidence of pressure ulcers.

The results support the importance of the knowledge of ward nurses and their decision-making. A total of 11 roles and functions of WOCNs were suggested to be meaningful. A total of 14 roles and functions of administrators were suggested to be meaningful. The educational background of nurse administrators tended to be related to the incidence of pressure ulcers, and the study of and training in nurse administration were related to the sense of influence among WOCNs.

Indicators of the quality of home care nursing for older persons

We investigated home care for older patients with pressure ulcers with expert visiting nurses. We created several indicators of the quality of home care nursing for older patients with pressure ulcers.

Publications

Nagano M, Ogata Y, Tokunaga K. Quality indicators for pressure ulcer management systems at hospitals from the perspective of relationship to

incidence (in Japanese). *Nihon Kango Hyoka Gakkaishi*. 2012; **2**: 9–18.

Adult Nursing

Shoko Fujino, *Professor*
Hiroaki Murata, *Assistant Professor*

Naomi Takashima, *Professor*
Ruka Seyama, *Assistant Professor*

General Summary

We have studied what material students have learned about clinical practice in adult nursing. We have investigated what experiences graduates had during clinical practice while they were students. We then found how to develop the nursing process and how to communicate with patients. We plan to reflect upon these results and apply them to our education curriculum.

Research Activities

Fujino investigated the effective touch techniques used by nurses for palliative care. She recorded interviews with 7 hospice nurses and 4 pain-control nurses about their touching of patients. The results were classified and described in 17 concepts and 11 categories. The nurses understood that touch was comforting and that they touch patients to

comfort them. This touching, which Fujino calls “caring touch,” is used to strengthen the relationship between nurses and patients and to ease suffering.

Takashima investigated the stress experience of patients who were admitted to the intensive care unit and had used a respirator for 24 hours or more. She also reported on the correlation between physical activity and hospital anxiety and depression, health-related quality of life, and the life condition 2 months after gastric resection.

Murata described the experiences of patients with acute respiratory failure receiving ventilatory support and continued research for the development of a nursing support program. She also started research on patients with delirium.

Seyama described the experiences of patients who were treated for cancer and the suggestion for nursing. She also continued research for the development of a care model that includes families.

Publications

Takashima N, Murata H. Quantitative and qualitative evaluation of the quality of life of patients with gastric cancer up to 2 months after gastrectomy (in Japanese). *Tokyo Jikeikai Ika Daigaku Zasshi*. 2013; **128**: 25-34.

Takashima N, Murata H, Watanabe C, Nonaka M, Ozone M, Nakata K, Mitsumori Y, Kashiwagi H. Correlation between Hospital anxiety and depression and QOL, life condition

after perioperative gastric resection (in Japanese). *Shokaki Shinshin Igaku*. 2012; **19**: 14-20.

Reviews and Books

Hayakawa K, Takano T, Takashima N, editors. ICU CCU Nursing (in Japanese). Tokyo: Igaku Shoin; 2013.

Gerontological Nursing

Miyoko Sakurai, *Professor*

Junko Kusachi, *Associate Professor*

General Summary

We created the visual educational DVD, “Assessment of Cases for Nursing, Volume 5: Nursing Cases of Patients with Stroke,” to help nursing students understand the nursing care of patients with stroke, who are often placed in the care of nursing students as part of practical training. We hope that this educational material will help nursing students to form an image when learning about caring for the elderly, which has traditionally been conducted with the use of simulated “paper patients.” In addition, a large number of exercises with simulated patients have been incorporated into the curriculum to improve nursing techniques for patients with dysphagia and decubitus ulcers.

Research Activities

We focused on the topic of “Investigation of an exercise program to support the independent activities of the elderly who require nursing care: the state of lower limb edema and related factors in long-term wheelchair users,” which has been incorporated into the edu-

cational curriculum. This year, we conducted a survey among wheelchair-using residents of healthcare facilities for the elderly regarding the state of lower-limb edema. In the future, we intend to investigate an exercise program designed to improve lower-limb edema on the basis of the physical exercise capabilities of the subjects and the time frame in which edema increases.

Mental Health and Psychiatric Nursing

Masashi Kawano, *Professor*

Junko Ishikawa, *Assistant Professor*

General Summary

Education and Research

In education, we have continued revising collaborative lectures, laboratories, and practical training for 5 years.

For freshmen, we gave lectures about the concepts and current issues of mental health.

For sophomores, we gave lectures about working with psychiatrists, psychiatric diagnosis, and nursing care for patients with psychiatric illnesses. We invited guest speakers who were mentally ill persons from the Kagayaki-kai to help students understand the features of mental illness. For juniors, education mainly involved laboratories about communication skills, interpersonal relationships, and the nursing process using DVDs produced by our department, with Kichijoji Hospital used for the subsequent practical training. The final examination we gave was not paper test but a practical examination involving roleplay. For junior students we trained at Kichijoji Hospital and on Ward 11E at The Jikei University Hospital. For senior year comprehensive practical training, we redefined the goals and objectives for a deeper understanding of and the use of skills for patients with acute psychiatric illnesses and forensic psychiatry at Tokyo Musashino Hospital and National Center Hospital, National Center of Neurology and Psychiatry.

In research, we finished a study comparing psychiatric healthcare for patients in rural areas between Japan and Thailand and a study of child and adolescent psychiatric care in Shimo-Itabashi, Toshima, and Nerima Wards of Tokyo. We continued our research on communication skills needed for undergraduate and graduate education in nursing. Other studies examined the utilization of recovery clubs by alcoholics and analyzed persons recovering from depression.

Evaluation

The department is in the fifth year of redefining the contents of education. We have received feedback from students of each class, and practical training was achieved. Feedback led to some effective changes. We were able to revise the entire contents of education. Producing and using a DVD contributed to classroom teaching and practical training and strengthened the relation between the hospital and the School of Nursing. It is important to continue applying the evaluations from students to improve lectures, labo-

ratories, and practical training. Next year we will use simulated patients for the final examination of junior students to enhance their clinical skills.

Research activity is needed to maintain a psychiatric mental-health-oriented approach. Applying research results to education is essential.

Child Nursing

Kiyo Hamanaka, *Professor*

Kinu Takahashi, *Associate Professor*

General Summary

In the field of education, educational systems have been strengthened to improve intramural and extramural training programs with Associate Professor Takahashi.

In the field of research, it is necessary to appropriately use the results of research on the educational support for hospitalized children. Regarding research on demands and expectations of members of the Association of Parents of Children with Incurable Diseases, analysis will be continuously performed, and the results will be reported, while continuously examining topics to contribute to clinical practice with its outcomes. Regarding research on the status of nurses advocating “children’s rights”, the status of education to promote “nursing ethics” and “children’s rights” in basic nursing education programs will be further analyzed in relation to the status of nursing, while studies are continuously performed to promote “children’s rights.” In addition, we have been working with other researchers in related fields and faculty staff members of other universities to perform collaborative studies.

Research Activities

Cooperation of educational and medical staff to provide educational support for hospitalized children

Hamanaka made presentations at meetings of the Japanese Society of Child Health Nursing and Nihon Ikuryo Association based on the results of last year’s analysis.

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

Hamanaka also performed a questionnaire survey involving members of the Association of Parents of Children with Incurable Diseases to clarify their use, demands, expectations, and feelings regarding Research into Treatment for Specific Chronic Child Diseases and other systems and support services.

Their answers to the questions of the Likert scale, focusing on problems and challenging issues regarding the above-mentioned research plan and children with Incurable Diseases, as well as difficulties when using such systems and support services and expectations for

them, were quantitatively analyzed after descriptive statistical procedures were implemented. Descriptive data obtained in this survey will be qualitatively analyzed.

Experience of receiving ethics education among nurses engaged in ethics education and the status of nurses advocating “children’s rights,” with a focus on nurses involved in pediatric nursing

Takahashi performed a questionnaire survey involving pediatric nurses from 6 pediatric, university, or general hospitals in the Kanto region to investigate the experience of receiving ethics education among nurses engaged in ethics education and the status of nurses advocating “children’s rights.”

The results of quantitative analysis obtained in this survey were reported at the 32nd Academic Conference of the Japan Academy of Nursing Science. The results of descriptive analysis will be reported at a meeting of the Japanese Society of Child Health Nursing.

Status of education to promote “nursing ethics” and “children’s rights” in basic nursing education programs

Following last year’s study, a questionnaire survey was performed involving nursing universities, colleges, and 3-year vocational schools throughout Japan to examine the status of education to promote “nursing ethics” and “children’s rights” in basic nursing education programs. The data obtained in this survey are being analyzed.

Maternity Nursing

Kimiko Kayashima, *Professor*

Yasuko Hososaka, *Associate Professor*

General Summary

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

Research Activities

Comparison of Internet use and sexual health problems among adolescents in Japan and China

We analyzed Internet use and sexual health problems among adolescents from online newspaper articles published from 2006 through 2008 in Japan and China. Child-related issues concerning access to dating Websites, installation of content-filtering software, and creation of rules for cell-phone use are seen as problematic in Japan, whereas Internet addiction is seen as a problematic mental illness in China. The need for parents and guardians to take interest in their child’s computer use has been reported. Although a trend towards monitoring and limiting Internet use by adolescents can be seen in both

countries, media literacy education is still necessary.

The relationship between health actions and knowledge of women in their first pregnancy and health guidance from their healthcare provider

We analyzed the relationship between health actions and knowledge of women in their first pregnancy with a focus on the first half of pregnancy and guidance from their healthcare practitioner. We found that health behaviors and knowledge scores increased along with satisfaction ratings in health guidance but decreased when the score for negative support from the guidance provider increased. Therefore, to link health guidance to behavior modification in pregnant women, it is important to increase satisfaction in health guidance and promote an approach conveying a receptive attitude.

Microbiological, immunological, and nutritional safety of breast milk

The microbiological and immunological safety of breast milk was investigated for various storage and thawing methods using breast milk obtained from 20 adult nursing mothers 1 month postpartum and 2 samples of artificial milk for neonates. The bacterial count was affected by the storage method. In the immunological investigation, analysis was performed using immunoglobulin A and lipase as indicators, and lipase levels were found to decrease significantly for all thawing methods.

Survey of current newborn cleaning care using a mixed-methods approach

A mixed-methods approach was used to survey current methods of newborn cleaning care at 256 maternity clinics across Japan. In this approach, questionnaires were administered about methods of newborn cleaning care in the early neonatal period, and interviews were conducted with 5 midwives on their thoughts about the provision of cleaning care. Responses showed that clinics usually did not perform any particular cleaning care on the day of birth. The most common method of cleaning care used was regular bathing from day 2 onwards. The selection of a newborn cleaning care method was not affected by the number of deliveries or staff members. Dry bathing was significantly more common in the Kanto region, and regular bathing was significantly more common in Kyushu. The following 5 categories were extracted from the interviews: cleaning care that focuses on the burden to the newborn, care that conveys the importance of the newborn to the health practitioner, evaluation of traditional bathing from both good and bad sides, a dry technique that reduces the burden to the newborn and gives the health practitioners more leeway, and fluctuations in cleaning care for newborns.

Publications

Fujihara S¹, Kayashima K, Shimizu Y¹, Nishino J¹ (¹Nagano Coll Nurs). Comparison of problems related to Internet use and sexuality among adolescents in Japan and China: Based on online newspaper articles in Japan and China between 2006 and 2008 (in Japanese). *Nihon Seikagaku-kai Zasshi*. 2012; **30**: 51–62.

Hososaka Y, Nukita H, Ito F, Ishii Y, Onishi A,

Isonishi S. Effects of different thawing methods on the immune and nutritional contents of expressed breast milk (in Japanese). *Tokyo Jikeikai Medical Journal*. 2012; **127**: 105–12.

Reviews and Books

Kayashima K. Sexual Health and Nursing (in

Japanese). *Nihon Seikagakukai Zasshi*. 2012; **30**: 3-10.

Kayashima K. Nursing of the adult woman (in

Japanese). In: Horiuchi S, editor. *Midwifery Lecture 5: Midwifery diagnosis & skill 1*. 5th ed. Tokyo: Igaku Shoin; 2013. p. 217-44.

Community Health Nursing

Junko Shimasawa, *Associate Professor*
Yoshiko Kubo, *Assistant Professor*

Ikuko Takahashi, *Assistant Professor*

General Summary

The faculty's research has been focused on: 1) public health nursing care to promote continued community life by patients with mental illnesses living at home, 2) infection control in the community, 3) development of a mental healthcare scale for occupational health nurses, and 4) research on educational evaluation.

Research Activities

Public health nursing care for mentally ill patients living at home

The purpose of this study was to elucidate the features of assistance provided by public health nursing care to promote the continued community life by patients with mental illnesses living at home. In this study, such assistance was considered to be support that promoted continued life in the community by a patient with a mental illness in a manner suitable for that patient.

Infection control in the community

We do research on hand hygiene among care staff in aged-care facilities. Hand hygiene is a basic measure to prevent infections. The purpose of this study was to obtain suggestions for improving hand hygiene.

Development of a mental healthcare scale for occupational health nurses

Occupational health nurses (OHNs), who perform mental healthcare at workplaces, require extensive knowledge, experience, and expertise. In addition, OHNs must develop problem-solving ability. The development of OHNs, however, often depends on individual effort because few workplaces in Japan provide organizational job-training programs. Compared with educational systems for public health nurses, systems for OHNs in each company are more difficult to establish. Hence, to maintain the quality of mental healthcare among workplaces, the purpose of this study was to develop a mental healthcare scale that can be provided to OHNs.

Evaluation of Community Health Nursing Practicum

This study aimed to evaluate how the improved Community Health Nursing Practicum II has influenced learning by nursing students at the university. The law regarding nursing,

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

Reviews and Books

Kubo Y. Basis occupational health nursing theory: Patricia Benner (in Japanese). *Sangyo*

Kango. 2012; **4**: 399-404.

Home Care Nursing

Motoko Kita, *Professor*
Hiroko Toyama, *Assistant Professor*

Reiko Yoshida, *Assistant Professor*

General Summary

In Home Care Nursing, the lecture-based “Introduction to Home Care Nursing” and practice-based “Methodology of Home Care Nursing” and “Clinical Practice in Home Care Nursing” have been provided since 2011 to effectively help students develop practical nursing care skills step by step with due consideration of the characteristics of home care nursing. This year, we researched course evaluations in accordance with each instructor’s area of interest.

Research Activities

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

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

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

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

A study on the development of the process to support elderly patients with dementia to return home following a stay in acute care hospitals

Recently, an increasing number of elderly patients with dementia are being admitted to acute-care hospitals for the treatment of other diseases. However, it is difficult to effectively support their subsequent discharge to home. As the first step to develop a model to resolve the difficulty in supporting elderly patients with dementia to return home after discharge from an acute-care hospital, we investigated the process involving nurses in charge of providing such support. This year, as a preparatory step, we examined the literature related to the research framework and translated documents on related theories.

Basic research (Vol. 1) for creating an information-sharing system for team-based home care

We performed basic research to extract information required for effective mutual cooperation between home care team members, including the patients' families, and sharing of information with other team members in remote areas. First, we identified information to be shared between the home care nurses and other team members and recognized that the information needs to be shared not only with family members and physicians, but also with other related professionals.

The effect of intervention with narrative approaches on the anticipatory grief of patients' families providing terminal care at home

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

Basic research on network development concerning the preventive long-term care support network

We believe that developing a network to identify patients in need of preventive long-term care through an effective use of regional resources in cooperation with regional groups, local volunteers, and various professionals is a new key to help solve issues concerning preventive long-term care. As basic research, we conducted interviews with subjects belonging to different groups (such as association officials, volunteers who provide telephone counseling services, district welfare officers, and the staff of the community general support centers) to assess the information, needs, and resources of regional groups

with regard to preventive long-term care and categorize the data and examine issues in accordance with regional characteristics.

Inspection/evaluation

From the course evaluation performed this year, we observed the effectiveness of the existing series of learning processes for practice-based classes focusing on the home care nursing process. On the other hand, we have also observed issues, such as that the number of patient cases introduced in the practice-based classes and the class system may require reassessment because of the increasing number of students and that the lecture-based classes suffer because of the poor comprehension of students. To resolve these issues, it is necessary to further improve the classes and continue course evaluation.

The research performed by the teaching staff covers important issues in home care nursing. We hope to facilitate mutual support in the field and further develop the research.

Publications

Yatsu H (Jpn Red Cross Coll Nurs), Kita M. Issues of the generalization in qualitative research (in Japanese). *Nihon Kango Kenkyu Gakkai Zasshi*. 2012; **35**: 53-4.

Kita M. Introduction to synthesis of the qualitative research evidence and the interpretive

approach in systematic review (in Japanese). *Kango Kenkyu*. 2012; **45**: 253-9.

Yatsu H (Jpn Red Cross Coll Nurs), Kita M. Can't be the findings of qualitative research generalized? : generalizability in qualitative research (in Japanese). *Kango Kenkyu*. 2012; **45**: 414-20.

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