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Introduction

It is my great pleasure to publish *Research Activities 2009*, which is a report on the scientific and educational activities at The Jikei University School of Medicine in 2009. We publish a Japanese version each year in parallel with this English-language version. This issue contains the research activities in the departments, institutes, and laboratories of the Medical Science Center at The Jikei University School of Medicine in 2009. In this issue, only selected papers published by each department, institute, and laboratory are listed at the end of each report owing to limitations of space. Similarly, the names of staff are limited to assistant professor and above.

Research Activities is a short summary of the annual research works at The Jikei University School of Medicine. I hope that *Research Activities* is used by people outside our university as well as by the faculty members of our university. Research and education are fundamental and important activities, as are medical services at attached hospitals of a medical university. As President of The Jikei University School of Medicine, I have been encouraging our faculty members to perform research works that, in particular, support medical treatment and disease prevention. The faculty members of clinical departments are working in the attached hospitals as physicians, which supports our university. Although they have little time to spend in laboratories due to their work at attached hospitals, publishing papers or reports is a very important activity for faculty members. Our attached hospitals have 2,600 beds and see 7,200 outpatients each day. This means that we are in a good position to accumulate clinical data, which can be used for clinical studies, including clinical trials. I would like to encourage our faculty members to publish papers regarding clinical studies as well as basic research works.

Our university also provides a laboratory for people who can perform basic research by themselves in the Research Center for Medical Sciences to promote basic research works.

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

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

Satoshi Kurihara
President
The Jikei University School of Medicine

December 7, 2010

Continuing Medical Education Center The Continuing Medical Education Committee

Toshiaki Abe, *Director*
Yashuo Toriumi
Katsuyoshi Tojo

Keizo Takagi
Akihiko Ohno

General Summary

The Continuing Medical Education (CME) Center was established in 1982 to commemorate the centennial of The Jikei University and to support the education of doctors outside the university hospital. Registered members consist of alumni throughout Japan, members of the local medical association, and doctors who have been approved by the Jikei CME Center. Members are allowed to use the facilities (video, library) of the CME 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 CME Center, and in scientific meetings and conferences held by the department.

Activities

1. Registered members: 237 (as of April 1, 2010)
Members using the CME Center: 147/year
Telephone service: 80 cases
2. The 30th summer seminar was held on August 8, 2009. Eighty-eight persons participated.
3. Monthly seminars were held on the second Saturday afternoons of the month in April, May, June, July, September, November, February, and March. Twenty-five to 30 persons attended each seminar.
4. The "CME Center News" is mailed monthly to registered members.

Center for Medical Education

Osamu Fukushima, *Director and Professor*
 Mariko Itsubo, *Professor*
 Sugino Oishi, *Professor*
 Hisashi Onoue, *Associate Professor*
 Kazunori Utsunomiya, *Associate Professor*
 Machiko Hirao, *Associate Professor*
 Yoshio Ishibashi, *Assistant Professor*
 Toshikazu Sakuyama, *Assistant Professor*

Naofumi Kimura, *Professor*
 Hideaki Kashiwagi, *Professor*
 Tetsuya Kawamura, *Associate Professor*
 Masato Matsushima, *Associate Professor*
 Mariko Nakamura, *Associate Professor*
 Nobuyuki Furutani, *Assistant Professor*
 Hiroyuki Takahashi, *Assistant Professor*

General Summary

The Office of Educational Development was founded in 1999. Staff members were recruited from the School of Medicine. Its main interests were analysis of medical education reports published by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), the Ministry of Health, Labour and Welfare, and medical associations; technical support of faculty, management of faculty development and education seminars; and the implementation of tutorials, objective structured clinical examinations (OSCEs), and community-based medical education programs in 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. The Center consists of the Office of Medical Education, the Office of Nursing Education, the Office of Postgraduate Clinical Training, and the Office of Educational Development. Furthermore, the secretariat was set up in the Center in April 2006. The Office of Medical Education contributed to revisions of the undergraduate curriculum, to implementation of OSCEs in years 4 and 5, and to faculty development programs (writing multiple-choice questions, educational techniques in the clinical setting, and rater training for OSCE). The Office of Nursing Education contributed to faculty development programs for nursing teachers (physical assessment training). The Office of Postgraduate Clinical Training supported the management of residency programs and the implementation of faculty development programs for attending doctors belonging to the 4 attached hospitals. The Office of Medical Development helped establish of an e-Learning system for students and healthcare providers in the community, managed the simulation center, and implemented several continuing learning courses (auscultation seminar and physical assessment training courses) for district nurses in the community.

Research Activities

1. We promoted the development of community-based medical education and offered continuing professional development courses to healthcare providers in the community with a Supporting Grant for Interuniversity Educational Program 2008 from MEXT. This project was a collaboration of 4 medical schools (The Jikei University, Showa

University, Toho University, and Tokyo Medical University). In the project, we promoted staff development programs for the technical staff of medical schools and the restructuring of the e-Learning system used by students of the 4 medical schools. We also produced new electronic educational materials for basic clinical procedures, tutorial materials, and clinicopathological conference materials supplied via an intranet system to our medical students and postgraduate trainees.

2. We promoted community-based medical education for undergraduate medical students and nursing students and continuing professional development programs for health-care providers in the community with a Supporting Grant for Distinctive University Educational 2007 from MEXT. We included family medicine practice as a clinical rotation for 5th year students and implemented primary care and selected clinical training at community hospitals for students in the 2nd through 6th years. Students learned about the good patient-physician relationship, psychosocial aspects of patients, multidisciplinary cooperation, and the management of patients with chronic diseases in the community. We held 4 auscultation seminars, 7 physical assessment seminars, and an e-Learning course about palliative care at home to district nurses concerned with in-home care curricula of medical and nursing schools. This project was completed this year. We invited 2 raters from King's College London and Tokyo Medical University to observe our activities. We published a final report.

3. We implement 6 interprofessional educational programs to medical students: Community Service for the Handicapped Program (year 1), Care for Severely Handicapped Children, and Support for Child Rearing in the Community Program (year 2), Health Care at Home Program (year 3), Working at a Hospital Program (year 4), and Workshop for Teamwork Building at a Hospital (year 5). We published the contents of these programs as a review (see below).

4. Workshop for Teamwork Building at a Hospital: We organized workshops held in April (Nishishimbashi), June (Daisann), July (Kashiwa), September (Nishishimbashi), October (Aoto), November (Daisan), December (Kashiwa), and January (Nishishimbashi).

5. Contribution to other institutions of higher education (Faculty Development lecture and workshop): Tokyo Medical Association (October), MEXT (July), Ministry of Health, Labour and Welfare (January), Kyorin University (May and October), Kitasato University (June), Tokai University (September), Tokushima University (October), Kumamoto University (December), and Toho University (March).

Reviews

Fukushima O. Jikei University School of Medicine: An interprofessional medical education program. In: Watababe H, Koizumi M, editors.

Advanced initiatives in interprofessional education in Japan. Tokyo: Springer; 2010. p.49-56.

Department of Anatomy (Gross Anatomy and Neuroanatomy)

Yoshinori Kawai, *Professor*

Mitsuyo Maeda, *Assistant Professor*

General Summary

Our department's research activities have focused on neuroanatomy and gross anatomy. In neuroanatomical research, the development and organization of neuronal networks are investigated to clarify brain functions 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 architecture and dynamics of microcircuits and their relationships. In gross anatomical research, the functional importance of variations in organ systems is explored using cadavers and animals.

Research Activities

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

Region- and size-specific neuronal organizations of the caudal nucleus of the tractus solitarius (cNTS) were investigated, after which excitatory and inhibitory synaptic input patterns onto specific cell types were investigated 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 GABAergic somata, small calbindin neurons constituted 42% of all cells. The medial subnucleus contained large numbers of glutamatergic, GABAergic, and catecholaminergic somata, and large tyrosine hydroxylase-containing cells constituted 13% of cells in this region. In total, small neurons ($<150 \mu\text{m}^2$) represented about 80% of the cell population in the cNTS. Predominant excitatory postsynaptic currents were observed in adult small neurons, whereas inhibitory postsynaptic currents were more evident in larger neurons, irrespective of subnuclear location. This distinct differentiation of postsynaptic current patterns was not evident in neonates. In adults GABAergic synapses were more frequently associated with dendrites of large catecholaminergic cells (73%) than with those of small calbindin-containing cells (10%). These results indicate that differential synaptic input patterns were developmentally established in distinct small and large neurons.

Local axonal arborization patterns of distinct neuronal types in the cNTS

Neurons in the cNTS are heterogeneous in 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 patterns of axonal branching behavior, cNTS cells could be classified into 2 groups: smaller cells ($94.1 \mu\text{m}^2$ in mean somal area range, $62\text{--}120 \mu\text{m}^2$; $n=22$) and larger cells ($245 \mu\text{m}^2$ in mean somal area; 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 no or few axon collaterals, suggesting their distinct roles as local-circuit neurons (or interneurons) and projection neurons, respectively. With regard to somatodendritic characteristics, the following correlations with cell size were found: smaller cells had larger form factors than did larger cells ($P<0.05$); and larger neurons had more extensive dendritic arborization, expressed by total dendritic length ($P<0.01$) and by the number of dendritic branching points ($P<0.01$), than did smaller cells. These findings suggest that small cNTS neurons contribute specifically to an integration of input information generated in the local circuits, whereas large neurons convey the integrated information to other autonomic brain regions.

Postnatal development of GABAergic axon terminals in the rat cNTS

The proper function of the brain depends on a precise arrangement of excitatory and inhibitory synapses. Although the cNTS plays a pivotal role in cardiorespiratory reflexes, we know little about the formation of the local neural network in the cNTS. In the present study, we focused on GABAergic axon terminals and used immunocytochemical methods to study postnatal changes in GABAergic synaptic organizations in the rat cNTS at both the light and electron-microscopic levels. The counting of synaptic and nonsynaptic GABAergic axon terminals revealed that the number of GABAergic axon terminals in the cNTS was constant until the second postnatal week and that GABAergic axon terminals were reorganized around postnatal day 10 (P10). Electron-microscopic observation revealed that most GABAergic axon terminals formed axosomatic synapses on neurons with smaller soma (smaller neurons) at P2 to P4 but that the number of axosomatic synapses decreased considerably after P8. Orphan GABAergic boutons were present specifically near somata of smaller neurons at P10, and the number of axodendritic synapses on thicker dendrites decreased gradually during postnatal development. These results show that GABAergic axon terminals detach from the somata of smaller neurons during the second postnatal week. Such morphologic changes in axon terminals could cause changes in electrophysiological activity and might contribute to the reorganization of the local network within the cNTS from the neonatal to the adult type. These postnatal changes in the cNTS local network might be required 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 disrupts normal circuit formation. This phenomenon has been extensively investigated in several neural systems, including the somatosensory system, but has not been observed in the visceral sensory system. Ultrastructural

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

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

Astrocytes are thought to be active participants in synaptic plasticity in the developing nervous system. Previous studies have suggested that the number of axosomatic synapses on the small cells of the rat cNTS decrease 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 positive for glial fibrillary acidic protein (GFAP), glutamate-aspartate transporter (GLAST), or glutamate transporter-1 (GLT-1) within the cNTS became more intensely stained as development proceeded. The GLAST-positive structures encompassed calbindin-positive small cell somata after P10. Electron microscopic observation indicated that astrocytic processes encompass the small cell soma, whereas the number of axosomatic synapses decreases as development proceeds. The timing for glial coverage of the small cell soma appears to be consistent with the decrease in axosomatic synapses on the small cells. These observations suggest that astrocytes participate actively in regulating the decrease of axosomatic synapses on small cells in the cNTS during postnatal development.

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

GABAergic neurons are major inhibitory interneurons that are widely distributed in the central nervous system. The cNTS, which plays a key role in respiratory, cardiovascular, and gastrointestinal function, contains GABAergic neurons that regulate neuronal firing. In the present study, GABAergic neuronal organization was analyzed in relation to the location of subnuclei in the mouse cNTS. According to the differential expression of glutamate decarboxylase (GAD) 67, vesicular glutamate transporter (VGLUT) 2, calbindin, and tyrosine hydroxylase (TH) messenger RNAs, the cNTS can be divided into 4 subnuclei: the subpostrema, dorsomedial, commissural, and medial subnuclei. The numerical density and size of soma in the 4 subnuclei were then quantified with an unbiased disector analysis. 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, prefer-

entially localized in the commissural and medial subnuclei, which represented $\geq 50\%$ of small cells in these subnuclei. Thus, the GABAergic small neurons were located around the 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 cNTS: Differences between dorsal and ventral subnuclei

Inhibitory axosomatic synapses can effectively suppress the excitability of postsynaptic cells. Therefore, to understand the maturation of information processing, the development of inhibitory axosomatic synapses should be examined. 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 drastically from P5 to P10. In the ventral subnuclei, the percentage of GAD-positive terminals on the soma, the percentage of small or large cell somata with synapses, and the axosomatic synapse density were maintained or increased from P5 to P10. Thus, the decrease in the number of inhibitory axosomatic synapses in the dorsal subnuclei might facilitate maturation of fine receptive areas for peripheral inputs, whereas the increase in the number of inhibitory axosomatic synapses in the ventral subnuclei might facilitate the establishment of an effective regulation system for cNTS output.

Publications

Tashiro Y, Okada T, Kawai Y. Postnatal development of axosomatic synapses in the rat nucleus tractus solitarius: Dorsal and ventral subnuclei differences. *Neurosci Lett* 2009;

450: 217–20.

Negishi Y, Kawai Y. Cell-location specific developmental rewiring in visceral sensory nucleus. *Neurosci Res* 2009; **65 Suppl:** 213.

Department of Anatomy (Histology and Embryology)

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Yasuyo Shigetani, *Assistant Professor*

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

General Summary

Our group is interested in the developmental and evolutionary aspects of human organs. By comparing organ development in humans and other vertebrates, we are attempting to reconstitute the evolutionary path that each of our organs has taken, at both the molecular and morphological levels, thus identifying fundamental molecular mechanisms that shape each organ.

Research Activities

Polypterus, a model animal for studying tetrapod evolution

The evolutionary transition from aquatic vertebrates to terrestrial tetrapods occurred around 370 million years ago in the late Devonian period. What changes in genome function caused such evolution and led to the development of new organ remains uncertain. We have been investigating the evolution of the tetrapod body plan using a primitive actinopterygian fish, *Polypterus senegalus*, with analyses of comparative anatomy, comparative developmental biology, and comparative genomics. In collaboration with Professor Asao Fujiyama of the National Institute of Genetics, we have performed expressed sequence tag analyses of *Polypterus* embryos at stages later than neurulation. We have determined nucleic acid sequences of more than 50,000 clones and annotated each sequence by tBlastn analyses.

Iron metabolism of a Progressive Ataxic Gait Mouse

The progressive ataxic gait mouse shows progressive severe ataxia of the hind limbs with a severe gait disorder. This ataxic mouse has the following characteristics: 1) ataxia appears 28 to 38 days after birth; 2) ataxia develops in approximately 25% of mice, indicating that the causative gene is autosomal recessive; 3) many vacuoles are found in the cerebellar medulla; and 4) heavy Fe^{3+} deposits are found in the liver and the kidney. Therefore, this mutant mouse was assumed to be a novel model for spinocerebellar degeneration, especially for Friedreich ataxia. We have used this mouse to investigate the intracellular localization of iron deposition in the liver and kidney, the degree of the iron deposition in the liver and kidney with age, and the messenger (m) RNA expression of frataxin and ceruloplasmin in the liver and the kidney. Iron deposits in the kidney were localized in the apical site of each cell of the proximal convoluted tubules. Ultrastructurally, electron dense depositions were found in the cytoplasm or partly in lysosomes in the cells of the liver and kidney, but not in mitochondria. The degree of iron deposition in both tissues became more severe with age. The mRNA level of frataxin in neural tissues, except for the cerebellum, and the kidney, did not differ

between normal and ataxic mice. Although the level of frataxin mRNA in the cerebellum was significantly lower in ataxic mice than in normal mice, the level in the liver was significantly higher in ataxic mice. The expression levels in the aged liver and kidney were lower than in younger organs. The mRNA level of ceruloplasmin in the liver was down-regulated with age, as seen with frataxin. However, the expression in the kidney was up-regulated with age, although its level in the kidney was 1/100th that in the liver. Expression in the kidney was significantly higher in aged ataxic female mice than in younger mice. These data suggest that, although this mutant mouse is not a model for Friedreich ataxia, it is a useful model for studying the relationship between neurodegeneration and iron metabolism.

A novel method for analyzing the pathophysiology of chronic diseases and application to the Fabry model mouse

In addition to genetic variation, epigenetics provides an additional layer of phenotypic variation that might mediate the relationship between genotype and internal and external environmental factors. This finding suggests that tissue-specific adaptive states to chronic exposure to internal and external environmental factors might be understood through the examination of tissue-specific epigenomes. A new method was designed to understand the cellular pathophysiology in chronic diseases by examining tissue-specific epigenomes. The method is combined with tissue-specific tag-exchange in H3.3, a histone H3 variant localized specifically in transcriptional active regions, and chip-on-chip or chip-seq assay using specific antibodies for the tag. This method will be used to analyze the pathophysiology of Fabry disease, a slowly progressive inherited metabolic disease.

This year, a conditional tag-exchange vector for producing knock-in mice expressing tagged H3.3 was constructed.

Molecular mechanism of trigeminal placode and ganglion development in the vertebrates

The trigeminal nerve is the largest cranial nerve, containing both sensory and motor neurons responsible primarily for sensation in the face and movement for mastication. The trigeminal ganglion comprises cells with 2 distinct origins: placode and neural crest cells. The mechanism of trigeminal ganglion development has been extensively studied in the chick; however, the molecular mechanism remains unknown. We investigated 1) the role of fibroblast growth factor (FGF) 8 signaling; and 2) unknown genes from an embryonic chick head ectoderm complementary DNA library in trigeminal nerve development. FGF8-positive regulation in the trigeminal placode repressed *Brn3a* expression as the earliest trigeminal placode marker, and removal of the isthmus, the source of FGF8 in the neural ectoderm, in contrast induced intensive expression of *Brn3a* and of another trigeminal placode marker, *Pax3*, suggesting that FGF8 signaling has a negative effect on trigeminal placode development. A pre-placode ectoderm that was recently reported arises at the edge of the neural plate; its explant can induce head placodes in culture by the addition of FGFs and bone morphogenetic protein 4. The pre-placode ectoderm induction system would help clarify the complex array of

genes involved in the programming of the trigeminal placode-specific identity. Genes of morphology, causable factors of diseases, and their related genes are isolated as complementary DNA clones, which may shed light upon the molecular mechanism that could bridge the gap between FGF8 signaling and the known trigeminal placode markers.

Study of the diaphragm: Development and acquisition

The diaphragm is a muscular membrane present only in mammals which separates body cavity to the thoracic cavity and the abdominal cavity. Its abnormal development might induce congenital diaphragmatic hernia, but the mechanism is unclear. To understand diaphragm development, we used the Pax3-GFP mouse, in which *Pax3* gene-expressing somites are labeled with green fluorescence protein (GFP), to observe myoblast migration in diaphragm development. We found GFP-expressing cells on the sides of the septum transversum at embryonic day 10.5, and at embryonic day 14.5 the diaphragm was covered by GFP-positive cells. Moreover, migration of myoblasts from the region of cervical somites 3 to 5, where the phrenic nerve develops, is observed at the midline of the body. These results may indicate that myoblasts migrate into the diaphragm from 2 different areas: cervical somites 3 to 5 and cervical somite 12. To understand in greater detail how the diaphragm developed in mammals, we will compare these observations to somite cell migration in other vertebrates.

Publications

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Department of Molecular Physiology

Yoshiki Umazume, *Professor*
Maki Yamaguchi, *Assistant Professor*

Shigeru Takemori, *Associate Professor*

General Summary

Our efforts have been concentrated on clarifying molecular mechanisms of the dynamic function of living cells from the aspect of protein-water interaction.

Research Activities

Model of intracellular water

Magnetic resonance (MR) images depict biological tissues on the basis of the differential transverse relaxation of water proton nuclear MR signals. Using skeletal muscle sarcomeres as a biological model of general biological tissues, we have shown that the relaxation process represents the restriction of water molecules from the surrounding tissue structure. To find nonbiological materials that restrict water molecules similarly to biological tissues, we tested nanoporous silica and reverse micelles. Water states in the nanoporous silica were similar to those in skeletal muscle sarcomeres, whereas those in reverse micelles were far less restricted by the interface of the micelles. These results confirmed that the restriction of water molecules is highly dependent on the nature of the interface between the water phase and the surrounding structure. Because the coexistence of myosin and actin in sarcomeres has been shown to strongly restrict sarcomeric water molecules, it is reasonable to consider that the contractile interaction between myosin and actin involves the transition of water molecules as a reservoir of free energy. We further hypothesized that biomolecular systems generally adopt similar strategies involving the restriction of neighboring water molecules to realize their physiological functions.

Evaluation of water state in living muscle from MR images for effective training

The obturator internus and externus muscles are important for stabilizing the hip joint. Patients with locomotor disabilities often need to train these muscles as part of their rehabilitation. To develop effective training methods, the activity of these muscles must be evaluated with MR because they are difficult to access with electromyography or ultrasonography. In this study, we evaluated the activity after exercise of the obturator internus and externus muscles with MR, which is generally considered effective from the view point of anatomical observation.

Before and immediately after 3 minutes of external rotation of the right hip joint at a frequency of 0.25 Hz, transverse relaxation processes were obtained with the multiecho Carr-Purcell-Meiboom-Gill sequence with 32 equidistant echos. The processes obtained from the obturator internus and externus muscles were assigned to 2 water groups with moderate time constants of transverse relaxation (T_2) out of 4 water groups

classified on the basis of data from dissected frog skeletal muscle. The water group of longer T_2 increased its relative content after exercise compared with the resting state in the obturator internus and externus muscles on both sides. The increase was especially prominent in the obturator externus muscle on the exercised side. These results are consistent with data from dissected frog muscle in the contracting state.

X-ray diffraction study of molecular mechanisms of cardiomyopathy due to troponin mutation

The E244D troponin T (TnT) mutant that causes hypertrophic cardiomyopathy increases the maximal tension of cardiac muscle fibers. To clarify how this mutation enhances the capacity to develop tension, we have been performing structural analysis by means of simulations of molecular dynamics and X-ray diffraction experiments.

Molecular dynamics at a steady state showed that the formation of electrostatic bonds between troponin I (TnI) and TnT involving the mutated amino acid was suppressed in E244D troponin, although its main-chain structure was essentially identical to that of the wild type. Molecular dynamics studies in a stressed condition showed that the mutation did not significantly affect the connectivity of TnI to TnT and suggested that the mutation has no significant effect on signal transduction from TnI to TnT.

To verify this hypothesis, we performed X-ray diffraction experiments on skinned muscle fibers in which endogenous troponin was replaced with wild-type or E244D TnT. The intensity of troponin reflection in both fibers suggested that the structural arrangement of E244D and wild-type TnT on thin filaments was similar. In fibers in which E244D TnT was introduced, the transition from the resting state to the contracting state was accompanied by a greater change in intensity of the myosin layer lines than in wild-type fibers; this finding indicates that a larger fraction of myosin heads are recruited to contractile interaction in E244D TnT fibers.

These results suggest that the rearrangement of local electrostatic bonding triggered by the mutation causes an abnormal interaction between TnT and tropomyosin and leads to a larger shift of tropomyosin. This larger shift of tropomyosin would allow increased recruitment of myosin heads to the actomyosin interaction so as to enhance the tension-development capacity in the E244D mutant.

Evaluation of the water state by viscosity measurement

Polyethylene glycol (PEG) narrows the lattice spacing of skinned skeletal muscle sarcomeres. Because the estimated size of PEG (molecular weight, 3,350) is several nanometers and the myofilament lattice spacing of 40 nm would allow PEG to penetrate, water within the sarcomere might have a different property to inhibit penetration by PEG. To test this hypothesis, the concentration of PEG within the sarcomere was estimated by measuring the specific gravity of myofibril suspensions from rabbit psoas muscle in the presence or absence of PEG. If PEG does not freely penetrate the sarcomere, the specific gravity of the supernatant after centrifugation of the myofibril suspension is expected to be larger than that before centrifugation.

The concentration of PEG within the sarcomere was half that outside the sarcomere, indicating that water within the sarcomere has a different property from the bulk water

surrounding the myofibrils. This finding supports our hypothesis.

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Department of Cell Physiology

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 Masato Konishi, *Visiting Professor*
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General Summary

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

Research Activities

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

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

Intracellular mechanisms of the increase in Ca^{2+} leak from ryanodine receptor by β -adrenoceptor stimulation in mouse cardiac muscle

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

Pathophysiology of cardiac muscle contraction in dilated cardiomyopathy

We are investigating, in collaboration with Kyushu University, the pathophysiology of

cardiac muscle contraction in a knock-in mouse model of inherited dilated cardiomyopathy (DCM). The administration of an angiotensin II type 1 receptor blocker, candesartan, was found to improve the survival rate in DCM mice, owing, presumably, to amelioration of myocardial fibrosis and electrocardiographic abnormalities.

Molecular mechanism of length-dependent activation in cardiac muscle

Cardiac sarcomeres produce greater active force in response to stretch, forming the basis of the Frank-Starling mechanism of the heart. In the present study, we explored the molecular mechanism of length-dependent activation by using skinned porcine ventricular muscle. We found that MgADP increased the Ca^{2+} sensitivity of force and the rate of the rise of active force, consistent with the increase in thin filament cooperative activation. MgADP attenuated length-dependent activation, with and without thin-filament reconstitution with the fast skeletal troponin complex. Conversely, inorganic phosphate decreased the Ca^{2+} sensitivity of force and the rate of rise of active force, consistent with the decrease in thin filament cooperative activation. Inorganic phosphate enhanced length-dependent activation, with and without skeletal troponin reconstitution. Linear regression analysis revealed that the magnitude of length-dependent activation was inversely correlated with the rate of rise of active force. These results were quantitatively simulated by a model that incorporates the Ca^{2+} -dependent “on-off” switching of the thin filament state and interfilament lattice spacing modulation. Our model analysis revealed that the cooperativity of the thin filament “on-off” switching, but not the Ca^{2+} -binding ability, determines the magnitude of the Frank-Starling effect. These findings demonstrate that the Frank-Starling relation is strongly influenced by thin filament cooperative activation.

Role of spontaneous sarcomeric oscillations in cardiac beat

Cardiac sarcomeres exhibit spontaneous rhythmic oscillations (SPOC) under partial activation states, namely, at $\text{pCa} \sim 6.0$ (Ca-SPOC), or with the coexistence of MgADP and inorganic phosphate under relaxing conditions (ADP-SPOC). We have reported that the period of SPOC (both Ca-SPOC and ADP-SPOC) in skinned myocardium correlates with that of the resting heart rate in various animal species (2005, 2006). In the present study, we analyzed the SPOC properties in rat neonatal cardiomyocytes expressing α -actinin-green fluorescent protein (GFP) in the Z-lines. We found that the Ca-SPOC occurred at $\text{pCa} \sim 6.0$ with a frequency of ~ 3 Hz after treatment with ionomycin. As found in adult cardiomyocytes, the sarcomeric oscillations consisted of quick lengthening and slow shortening during Ca-SPOC. In untreated neonatal myocytes an increase in the frequency of electrical stimuli to the physiological level (i. e., 3-5 Hz) caused a phase shift of shortening and relengthening due to enhancement of the relengthening speed, resulting in the waveform being similar to that observed during SPOC in ionomycin-treated cardiomyocytes. These results suggest that the intrinsic auto-oscillatory properties of sarcomeres may contribute to the regulation of cardiac beat *in vivo*.

Single sarcomere imaging in the heart in vivo

Numerous studies have been performed in tissues and cells to clarify the molecular mechanisms of myocardial contraction. However, because of differences between *in vitro* and *in vivo* conditions, the dynamics of myocardial sarcomere contractions in living animals is not understood. In the present study, we developed a novel system allowing for real-time imaging of single sarcomeres in the living heart. Male Wistar rats were anesthetized with pentobarbital sodium, and median sternotomy was performed under artificial ventilation. To visualize the Z-discs quantum dots (Qdots) were conjugated with anti- α -actinin antibodies and then transfected to the surface of the epicardium of the beating heart. An electron microscopic study confirmed the presence of Qdots in and around the T-tubules and Z-discs in the myocardial cells of the left ventricular wall. Consistent with this finding, a striated pattern of Qdots ($\sim 2\text{-}\mu\text{m}$ spacing) in the heart was observed with fluorescence microscopy. Furthermore, to visualize Z-discs at a higher resolution, we engineered GFP- α -actinin incorporated into adenoviral vectors. We are now performing real-time imaging of single sarcomeres by using Qdots and GFP in the beating heart of the rat.

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Department of Biochemistry

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Research Activities

Cancer research

1. Earlier diagnosis and therapy are the most effective methods for improvement of the prevention and the prognosis of the cancer. This year, to establish a effective, noninvasive *in vivo* system for detecting small tumors by means of molecular targeting/imaging methods, tenascin-C, a molecule that promotes tumor-cell motility and metastasis and serves as a prognostic indicator, was selected as the target molecule for cancer detection. The ultimate goal of our study is to develop a noninvasive method of early cancer diagnosis with antibodies against tenascin-C. To achieve this goal, the usefulness of antibodies against human tenascin-C was first determined in several human cancer cell lines by means of immunocytologic studies with antibodies labeled with Alexa488. These studies showed focal distribution of intracellular tenascin-C fluorescence with fine intercellular deposits among piled-up cells rather than among cells in a monolayer sheet.

Incubation of A431 cells in a spheroid culture, a conventional 3-dimensional culture method, or in a radial flow bioreactor (RFB), another 3-dimensional culture system that provides tissue architecture and molecular function mimicking the *in vivo* environment, resulted in upregulation of tenascin-C messenger RNA and protein with simultaneous downregulation of E-cadherin and overexpression of vimentin, as in the epithelial-mesenchymal transition, with significant accumulation of transforming growth factor beta 1 in conditioned media. The sequential change of molecules related to the epithelial-mesenchymal transition was also recognized in tumor cells transplanted into nude mice. These results confirm that tenascin-C is an appropriate marker for cancer detection in an *in vivo* environment.

Clinicopathologic analysis of a large series of patients with endometrial carcinoma also showed the significant correlation of tenascin-C expression with various histopathologic risk factors for cancer. To select suitable antibodies for *in vivo* imaging, we first examined whether the fluorescence-labeling procedures reduced anti-tenascin-C antibody titers; we found that the abilities of several labeled antibodies to recognize tenascin-C decreased 10% to 30% compared with those of intact antibodies.

The tissue distribution and tumor-targeting ability of Alexa488-labeled anti-tenascin-C antibodies were analyzed in tumor-bearing nude mice. The tumor: blood ratio was significantly elevated 48 hours after administration. In *in vivo* imaging, anti-tenascin-C antibodies coupled with infrared fluorescence accumulated gradually and peaked 48 hours after administration, and the strongest fluorescent intensity was detectable. Fluorescence intensity sufficient for imaging could be detected up to 7 days later.

To produce a large quantity of antibodies, antibody-producing hybridomas were altered to proliferate under serum-free conditions, which allow the antibodies to be purified

more easily. The hybridomas produced 10 to 20 mg of functionally active antibodies within 3 weeks of culture, by means of culturing methods using either a BD Falcon CELLline CL-1000 flask (BD, Franklin Lakes, NJ) or an RFB. This project showed that antibodies against CD147 or tenascin-C were useful *in vivo* molecular targeting tools.

2. Glucose metabolism is another target for cancer chemoprevention. CD147 is the accessory subunit of a heteromeric lactate transporter, monocarboxylate transporter (MCT), which is member of the solute carrier 16 (SLC16) family of solute transporters. The MCTs transport lactate across the plasma membrane, and the CD147-MCT interaction is required for MCT activity and for trafficking to the plasma membrane. Three-bromopyruvate (3-BrPA), a pyruvate/lactate analog, is a potent glycolytic inhibitor and a candidate anticancer agent. To determine the transporters involved in the cellular influx of 3-BrPA, the role of MCTs was examined. It is of interest that resistance to 3-BrPA was found when PC-3 cells were transfected with the small interfering RNA of only MCT1 among those of the SLC16 family. PC-3 cells pretreated with MCT1 inhibitors were also resistant to 3-BrPA. Furthermore, short hairpin RNA expression vectors specific for CD147 reduced the sensitivity of PC-3 cells against 3-BrPA, suggesting that the MCT1-CD147 complex is essential for 3-BrPA uptake. Our recent study has demonstrated that 3-BrPA is transported into PC-3 cells through a CD147-MCT1 heteromeric lactate transporter complex and promotes cell death. The cytotoxic activity of 3-BrPA against several cancer cell lines was enhanced under hypoxic conditions. In this microenvironment, the expression levels of CD147 and MCT1 increased compared with those under normoxic condition. These findings indicate that 3-BrPA is a potentially useful chemotherapeutic agent for hypoxic tumor cells that are preferentially resistant to chemotherapy.

3. Resistance of tumor cells to chemotherapeutic agents is a serious obstacle in cancer therapy. A conjugate of doxorubicin and glutathione *via* glutaraldehyde (GSH-DXR) strongly inhibited the glutathione *S*-transferase (GST) activity of rat hepatoma cells and all human tumor cells tested. The mechanism of action of GSH-DXR was the induction of apoptosis *via* activation of c-Jun N-terminal kinase by the binding of GSH-DXR to the active center of the GSTP1-1 enzyme, inducing cytochrome c release from mitochondria to the cytosol, caspase-3 activation, and DNA fragmentation. GSG-DXR encapsulated in an anti-CD147 IgG-coupled immunoliposome showed selective and superior anticancer effects, *via* a tumor-targeting mechanism, compared with free GSH-DXR, an empty liposome, or DXR encapsulated liposome.

4. Six cell lines with epoxomicin resistance were established. The epoxomicin-resistant cell lines are reliable tools for the therapeutic evaluation of proteasome inhibitors in preclinical trials. Moreover, these cell lines may also be useful for clarifying mechanisms of resistance to proteasome inhibitors and examining a wide variety of proteasomal functions. This year, the relation between E-cadherin expression and proteasomal inhibition was analyzed. In an epoxomicin-resistant human endometrial carcinoma cell line, Ishikawa variant, E-cadherin gene (CDH1) expression was suppressed *via* overexpression of ZEB1, a transcriptional repressor of E-cadherin. Further studies are now in progress.

5. Regulatory mechanisms of transcriptional co-activator with PDZ-binding motif (TAZ) linked to fibroblast growth factor (FGF)/receptor signaling, which plays an essential role in ossification, were determined with osteoblast-like MC3T3-E1 cells. We found that FGF-2, which inhibits bone mineralization and stimulates cell proliferation, reversibly reduced TAZ protein expression levels in MC3T3-E1 cells. A proteasome inhibitor, bortezomib, has recently been used to treat multiple myeloma. Bortezomib strongly induces the differentiation of osteoblasts and the activation of the transcription factor Runx2. However, the mechanism of osteoblast differentiation mediated by bortezomib remains unclear. Our previous report demonstrated that expression of TAZ, acting as a transcriptional coactivator of Runx2, was reduced in MC3T3-E1 cells after treatment with FGF-2. Our recent experiment has shown that bortezomib reverses the effects of FGF-2; namely, bortezomib inhibits FGF-2-induced reduction of TAZ and consequently stimulates osteogenic differentiation independently of the proteasome-inhibiting effects. These findings strongly suggest a possible mechanism for the observed bone-regenerating activity of bortezomib in patients with multiple myeloma, in addition to its anticancer activity.

Other research

1. Pharmaceutical plasma products play important roles in controlling many disorders. However, plasma products are often associated with numerous risks, such as the transmission of known and unknown infections, because they are manufactured from donated blood. The safe and efficient supply of such pharmaceutical plasma products is important. We have proposed and planned a project to produce a large quantity of plasma products with high-quality human albumin and fibrinogen using a well-defined human hepatocyte cell line cultivated in an RFB. This year, the quality and production ability of products, such as albumin and fibrinogen, were determined.

2. With methods to purify and identify ubiquitinated proteins in biological materials, several ubiquitin-protein conjugates in Tris-saline-soluble and Tris-saline-insoluble but 2% sodium dodecylsulfate (SDS)-soluble fractions were analyzed from cadmium-exposed human proximal tubular HK-2 cells. The treatment of HK-2 cells with sub-lethal concentrations of cadmium augmented water-insoluble but SDS-soluble ubiquitin-protein conjugates and rendered transcription factor STAT6 insoluble and, thus, decreased in ubiquitin target molecules in normal size in determined by SDS-PAGE. Our study confirmed that the cadmium-induced insolubilization of STAT6 was due to oxidative modifications of cysteine residues, including Cys384. These results may indicate a fundamental protective effect of STAT6 against cadmium toxicity. Therefore, structural changes in cellular proteins and the resulting loss of function are presumed to be involved in the expression of heavy metal toxicity.

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Department of Molecular Biology

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General Summary

Polyamines (putrescine, spermidine, and spermine) are ubiquitous biogenic amines present in all cells. The cellular polyamines mainly bind to RNA and DNA to stabilize them and to control their function and, therefore, are indispensable for cell proliferation. Polyamines also regulate various cellular processes, such as apoptosis and signaling through the *N*-methyl-D-aspartate receptors. The concentration of cellular polyamines is regulated by a family of proteins, termed antizymes (AZs). These AZs are expressed and induced through a unique mechanism, translational frameshifting, and negatively regulate cellular polyamine levels by accelerating the degradation of ornithine decarboxylase, a key enzyme for polyamine biosynthesis, and by inhibiting cellular polyamine uptake. Three AZ isoforms (AZ1–3) are conserved among mammals. Our goal is to clarify the mechanism and biological significance of the polyamine regulatory system, particularly the roles of the proteins involved.

Research Activities

Hematopoietic disturbance of AZ1-knockout mice

AZ1-deficient mouse embryos show hematopoietic disturbances and consequent severe anemia resulting in partial embryonic death. Our previous results suggested that the early erythroid progenitors are reduced in the livers of AZ1-deficient embryos. To further determine the hematopoietic steps affected, we analyzed embryonic liver cells with fluorescence-activated cell sorting (FACS) and colony formation assay. The FACS analysis revealed increases in hematopoietic stem cells and the early erythroid progenitor cells in the livers of AZ1-knockout embryos, indicating that the migration of early hematopoietic cells into the fetal liver is not impaired. On the colony formation assay, the mixed colonies decreased in the livers of AZ1-knockout embryos. The mixed colony corresponds to the common myeloid progenitor (CMP), a precursor of erythroid and myeloid lineages, and to the multipotent progenitor (MPP), a further precursor of CMP. Thus, the results suggest that the differentiation either from hematopoietic stem cells to MPP or from MPP to CMP is impaired in AZ1-deficient mice.

Defensive role of AZ1 against over-intake of polyamines

To assess the defensive role of AZ1 against over-intake of polyamines, adult AZ1-deficient knockout mice and control mice were fed composite meals containing a high dose of polyamines (25 times that of normal meals) for 2 weeks. As previously observed, AZ1-deficient mice tended to be leaner than the control mice at the start of the feeding experiment, but the changes in body weight did not differ between the groups

during the experiment. Blood levels of putrescine, spermidine, and spermine were higher in the AZ1-deficient mice than in the control mice before feeding, but spermine levels decreased markedly in the AZ1-deficient mice after day 7 of the high-polyamine feeding. Urine excretion of polyamines increased in both AZ1-deficient and the control mice fed high-polyamine meals until day 7. On day 14, polyamine excretion was still increasing in the AZ1-deficient mice but had decreased in control mice. Urinary levels of acetylated polyamines kept increasing with the high-polyamine diet, particularly in AZ1-deficient mice. These results suggest that, in wild-type mice absorption of polyamines is repressed by AZ1 when high doses of polyamines are ingested, whereas in AZ1-deficient mice, the lack of repression is compensated for by the acetylation of polyamines and the suppression of spermine synthesis.

AZ2 accelerates c-Myc degradation

We have found with yeast two-hybrid analysis that AZ2, but not AZ1, binds to cerebellar degeneration-related protein 2, which is a c-Myc-interacting protein implicated in paraneoplastic cerebellar degeneration. During the experiments, we found through pull-down assays that AZ2 directly interacts with c-Myc in cultured mammalian cells. Both AZ2 and c-Myc, tagged with fluorescent proteins, co-localized in the nucleus. AZ1 weakly interacted and partially co-localized with c-Myc. We then investigated the effect of AZ2 on the degradation of c-Myc. Overexpressed, tagged c-Myc, as well as the endogenous c-Myc, was degraded more rapidly in the cells transfected with AZ2 than in control cells. The acceleration of c-Myc degradation was inhibited by a proteasome inhibitor, MG132. The effect of AZ2 was not observed in a cell-free system. Finally we found that the addition of putrescine to cultured mammalian cells accelerated the degradation of endogenous c-Myc. These results suggest that an increase in cellular polyamines induces AZ2 and negatively regulates c-Myc through the acceleration of degradation by cellular 26S proteasomes.

Analyses of RNA aptamers against spermine

RNA aptamers are functional RNAs that have a high affinity for their biomolecular targets and can distinguish similar chemical structures. Last year we isolated RNA aptamers with a high affinity against spermine (spermine aptamers). To determine the spermine-binding regions of the aptamers, we performed mutational and domain exchange analysis between a spermine aptamer and another RNA aptamer that has a similar conformation but does not bind spermine. Multiple binding regions for spermine were determined on the spermine aptamer. Kinetic analysis between the spermine aptamer and spermine were also performed with the surface plasmon resonance technique. However, we could not determine the binding kinetics because of nonspecific interaction with each electrostatic region. Other methods are needed for analyzing the binding between the spermine aptamers and spermine.

A protein enhancing AZ frameshifting

We have been seeking cellular proteins that modulate translational frameshifting and found that transient expression of heterogeneous nuclear ribonucleoprotein A1-like

(hnRNP A1L) enhances the efficiency of frameshifting by 2 times over that of a co-expressed dual-luciferase reporter construct encoding the AZ1 frameshifting signal. A close homologue of hnRNP A1L, hnRNP A1, which is known to interact with various nucleic acids both in the cytosol and the nucleus, had no effect on the frameshift efficiency. The proteins hnRNP A1 and hnRNP A1L comprise 320 amino acid residues and have 11 amino acid differences. The different amino acids are spread over the molecules and on some domains, such as 2 RNA-recognition motifs, the RGG box and the M9 signal sequence. Domain substitution analysis with a series of chimeric constructs between hnRNP A1 and A1L showed that the 2 RNA recognition motifs of hnRNP A1L are important for the frameshifting-enhancing effect.

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Department of Pharmacology

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General Summary

The research interests in the Department of Pharmacology include the following.

- 1) Synaptic transmission and its modulation in the basal ganglia and basal forebrain (Toshihiko Momiyama)
- 2) Effects of new quinolones on body temperature and blood pressure *in vivo* (Seiji Hori)
- 3) Respiratory neural activities in *Xenopus* (Naofumi Kimura)
- 4) Design of secretory proteins (Yuji Ohno)
- 5) Purinergic receptors on human adrenocortical cells and mechanisms of histamine release modification by purinergic receptors in human lung mast cells (Haruhisa Nishi)
- 6) Cerebrocerebellar interactions (Taro Ishikawa)
- 7) Cardiovascular endocrinology (Keiichi Ikeda)
- 8) The basic mechanism of a ketogenic diet: purinergic autocrine regulation of CA3 pyramidal neurons (Masahito Kawamura)

Research Activities

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

Electrophysiological studies using slice patch-clamp recording are performed to analyze synaptic transmission, its modulation by neuromodulators, and their developmental change in the nigrostriatal or mesolimbic dopaminergic system, and the cholinergic system in the basal forebrain. These systems are involved in various psychological functions as well as their disorders, including Parkinson's disease and Alzheimer's disease. The regulation of output from these systems to the cerebral cortex is also studied.

Another issue is the regeneration of synapses and local circuits after basal ganglia-related disorders. In these disorders, electrophysiological, morphological, and behavioral studies are performed to clarify the mechanisms and time course of reconstruction of synaptic organization and transmission and the functions of whole animals in Parkinson's disease model rats or cerebral ischemia model rats.

Effects of new quinolones on body temperature and blood pressure in vivo (collaborative study with Department of Practical Pharmacy, Keio University School of Pharmacy)

We studied the effect of new quinolones on body temperature in mice. We showed that new quinolones decreased body temperature in rats in a dose-dependent manner.

Gatifloxacin had potent body temperature-decreasing activity, but levofloxacin had weak activity. Our *in vivo* results suggest that each new quinolone has its own body temperature-decreasing activity and that we should be aware of this activity, especially when a new quinolone is used for a patient with compromised renal function.

We also studied the effects of new quinolones on blood pressure in rats. In our *in-vivo* experiments, new quinolones decreased blood pressure in a dose-dependent manner. Garenoxacin had stronger activity, and levofloxacin had weaker activity. This *in vivo* result suggests that each new quinolone has potent blood pressure-decreasing activity. We are studying the precise mechanisms of these activities of new quinolones.

Respiratory neural activities in Xenopus

Aquatic pipid frogs have some interesting characteristics from the viewpoint of comparative respiration physiology. Unlike other anurans, pipids lack the buccal ventilatory cycle, exhale air from the lung before aspirating air into the buccal cavity, and have inherent muscles that may be homologous to the mammalian diaphragm. To study the mechanism of the lack of the buccal cycle, respiratory motor activities were recorded from the isolated brainstem-spinal cord preparations of *Xenopus laevis*. The intermittent lung ventilation-like burst complexes occurred in cranial nerves V and X, the hypoglossal nerve, and the third spinal nerve innervating the “diaphragm.” The buccal ventilation-like activity occurred in cranial nerves V and X, but did not appear in the hypoglossal nerve or the third spinal nerve of *Xenopus*. These results suggest that the brainstem of *Xenopus* is capable of buccal oscillation but partly lacks the motor output.

Design of secretory proteins

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

Purinergic receptors on human adrenocortical cells and mechanisms of histamine release modification by purinergic receptors in human lung mast cells

The expression and pharmacological function of purinergic receptors in H295R, a cell line derived from human adrenocortical cells, were studied as a model of these receptors in human adrenocortical cells. H295R expressed G-protein coupled P2Y receptors, suggesting that human adrenocortical cells express multiple purinergic receptors linked to their steroidogenic function. These results suggest that Ca^{2+} influx is important for the steroidogenesis of H295R cells.

In collaboration with Dr. E.S. Schulman of Drexel University (Philadelphia, PA, USA),

we synthesized several plasmids with the construct of short hairpin RNA (shRNA)-interference for the knock-down of histidine decarboxylase and P2Y2 receptors (a subtype of P2Y receptors). The gene knock-down systems were transfected with a lentivirus to LAD2 cells, a cell line derived from human mast cells. The results suggest that multiple purinergic receptors are related to the modification of IgE receptor-mediated histamine release in human lung mast cells.

Cerebrocerebellar interactions

The pontine nuclei in the brainstem receive signals from the cerebral cortex and relay them to the cerebellum. We have started a project that is aimed to clarify the rules of signal conversion in the pontine nuclei and the underlying cellular mechanisms through a combination of *in vivo* (anaesthetized animal) and *in vitro* (brain slice) experimental systems. Using the *in vitro* system, we made patch-clamp recordings from principal cells, which are excitatory projecting neurons, in slices of the pontine nuclei and investigated cellular properties, including the spontaneous firing rate and current-voltage relationships. Spontaneous and stimulation-evoked excitatory synaptic currents were also recorded, and their facilitation and depression patterns were analyzed. While developing the *in vivo* experimental system, we could make patch-clamp recordings from the pontine nuclei of anaesthetized animals via a ventral approach.

Cardiovascular endocrinology

We have been studying cardiovascular endocrinology in 2009. On the basis of a previous study of the secretion of adrenocorticotrophic hormone from the hypertensive heart and the expression of corticotropin-releasing hormone type 2 receptor in cardiomyocytes, we studied the secretion of adrenocorticotrophic hormone from cardiomyocytes using the HL-1 mouse atrial cardiomyocyte cell line.

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

A ketogenic (low-carbohydrate, high-fat) diet has successfully been used to treat pediatric and medically refractory epilepsy. The mechanisms underlying the success of ketogenic diet therapy, however, are not well understood. A ketogenic diet has been reported to increase ATP concentrations in the central nervous system and to cause mild hypoglycemia. To clarify the role of extracellular purines underlying the anticonvulsant effect of the ketogenic diet, whole-cell voltage clamp recordings were made from CA3 pyramidal neurons in acute hippocampal slices of rats. In conditions of reduced extracellular glucose and high intracellular ATP concentrations, CA3 pyramidal neurons hyperpolarize themselves via direct ATP release through pannexin-1 channels with the subsequent activation of adenosine A1 receptors. This autocrine regulation might be an important mechanism underlying the success of a ketogenic diet.

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Department of Pathology

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General summary

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

Research Activities

Pathology of the liver

The proliferating bile ductules of the liver in obstructive jaundice were examined with immunohistochemistry for CK7, CK19, and a human hepatocyte marker (Hepatocyte) to elucidate the origin of the ductules. Immunohistochemical double-staining for CK7 and Hepatocyte was also performed. Most liver cells were positive for Hepatocyte but not for CK7, whereas the epithelial cells of bile ducts and ductules were positive for CK7 but not for Hepatocyte. In cases of prolonged jaundice, many liver cells expressed both CK7 and Hepatocyte. These findings indicate that a part of liver cells showing such coexpression could change directly into epithelial cells of the proliferating bile ductules. We concluded that the proliferating bile ductules might derive from hepatocytes by direct transdifferentiation, not by indirect transdifferentiation matapasia.

The lymphatic channels of portal areas in the normal liver were examined by histologic reconstruction using serial sections and immunohistochemical studies for a lymph-vessel marker (D2-40). The lymphatic channels were divided into 2 systems on the basis of function. One is a system of lymphatic channels that run along arteries. Its role is to collect surplus interstitial fluid that leaks through the arterial wall to decrease arterial pressure. The role of the other system is to collect various substances produced in the portal areas and transport them to lymph nodes.

We continued to study the role of oxidative stress in the pathogenesis and evolution of nonalcoholic fatty liver disease. The distribution and intensity of oxidative stress markers were immunohistochemically examined with sections cut from paraffin blocks without degreasing. The results showed that both the nuclei and cytoplasm of liver cells were extensively positive for DNA oxidative stress markers (8-hydroxydeoxyguanosine and thymidineglycol). On the other hand, liver cells were negative for a fat oxidative stress marker (hexanoyl-lysine).

The cross-sectional area of the parenchyma, portal tracts, portal veins, arteries, and bile

ducts and the cross-sectional area of parenchyma per portal area in normal liver were determined with morphometry. The area ratio of the parenchymal portion was 93.5%. The mean sectional area of the parenchymal area per portal tract was 0.10 cm². The figure was constant regardless of age.

Nonalcoholic steatohepatitis associated with hepatocellular carcinoma was studied.

Renal pathology

Mitochondrial nephropathy with genetically proven mitochondrial disease was examined with light and electron microscopy. Granular swollen epithelial cells (GSECs) were found among the distal and collecting tubules. Ultrastructural analysis showed accumulation of abnormally shaped mitochondria in GSECs. Genetic analysis of GSECs revealed that their mutant load was significantly greater than that of normal-appearing epithelial cells within the same sample.

Histopathological variables listed in the Oxford international classification and the Japanese classification of IgA nephropathy were further investigated to improve interobserver reproducibility. On the basis of the results, the histological classification was revised.

Histologic reevaluation of specimens of 1,545 renal cell carcinomas collected in the department was continued with revised general rules for clinical and pathological studies of renal cell carcinoma. The file contains 5 cases associated with polycystic kidney, 4 cases with bronchial metastasis, and 13 cases of bilateral renal cell carcinoma.

The relationship between renal function and pathologic findings of renal biopsy specimens was examined in various renal diseases and transplanted kidneys.

Gastrointestinal pathology

Risk factors for metastasis of submucosal invasive colon cancers to lymph nodes were investigated using 203 surgically resected submucosal invasive colon cancers. In particular, vessel permeation and budding of cancer cells as risk factors were analyzed in this study. Special stains such as CD31, CD34, and D2-40 immunostains and elastica van Gieson stain were used to evaluate blood and lymphatic vessel permeation. Multivariate analysis showed that lymphatic permeation and venous permeation were significant risk factors (odds ratios of 3.6 and 7.5, respectively). Univariate logistic analysis revealed that budding was related to lymph node metastasis, lymphatic permeation, and venous permeation (odds ratios of 3.4, 3.9 and 2.6, respectively). These results suggest that budding is deeply involved in lymphatic and venous permeation. In addition, a likelihood-ratio test showed no significant differences between a model that included budding and a model that excluded budding. These results further suggest that vessel permeation is the best risk factor for predicting lymph node metastasis.

Urogenital pathology

We continued a pathologic study of prostatic cancer. Akt is a major protein of the intracytoplasmic signaling system. Expression of Akt and phosphorylated Akt was examined immunohistochemically in cells of precancerous tissue of the prostate, microscopic prostate cancer, and clinical prostate cancer. The results suggest that the

expression of phosphorylated Akt is related to clinical stage, cancer invasion of the seminal vesicle, and a high level of prostate-specific antigen.

A total of 716 testicular tumors collected in the department were studied clinicopathologically and immunohistochemically. There were 5 cases of spermatocytic seminoma, 67 cases of tumors greater than 100 mm in diameter, 11 cases of bilateral tumors, and 13 cases of adenomatoid tumors.

Gynecological pathology

Early hydatidiform moles with a gestational age of less than 12 weeks were examined to establish histologic criteria.

Breast pathology

Breast tumors on the borderline between benignancy and malignancy were collected and analyzed clinicopathologically. The results showed that neuroendocrine ductal carcinoma in situ is difficult to diagnose.

Overexpression of human epidermal growth factor receptor (HER2) was evaluated immunohistochemically (Hercep test), and HER2 gene amplification was simultaneously determined with fluorescence in situ hybridization (FISH) in breast cancer. Three of 39 cases showed a discrepancy between overexpression on immunohistochemical studies and gene amplification by means of FISH.

Oncology

Genetic analysis of lung and liver cancers was continued to identify candidate susceptibility inhibitor genes that play an important role in carcinogenesis and progression.

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Department of Virology

Kazuhiro Kondo, *Professor*

General Summary

Human herpesvirus (HHV) 6, which can establish lifelong latent infections of hosts, is frequently reactivated. We are studying the molecular mechanisms of the latency and pathogenesis of HHV-6. Additionally, we are attempting to apply HHV-6 and HHV-7 to study of the mechanism of fatigue and to their use as viral vectors for gene therapy. Fatigue is an indispensable biological alarm to avoid the exhaustive state caused by severe stress and overwork, which may also induce a variety of diseases. We have investigated the molecular mechanism of herpesvirus reactivation, which is stimulated by fatigue, and identified the molecule that can induce viral reactivation during fatigue. Using our understanding of herpesvirus reactivation, we have developed a method for measuring the accumulation of fatigue by determining the amount of HHV, which is reactivated and released into saliva.

Research Activities

Development of a method for measuring accumulation of fatigue

The accumulation of fatigue causes many serious problems, such as death from overwork, suicide, and lifestyle-related diseases. However, scientific and medical studies of fatigue are not as advanced as those of other subjects, and not even a partial solution has been obtained for problems caused by fatigue in the health of individuals and society as a whole. The main reason for the delay in studies of fatigue and the development of methods for preventing and treating it is that a method for objectively measuring fatigue has not been proposed. In particular, a method for measuring the accumulation of fatigue or medium- and long-term continuous fatigue, which most severely affects life and health, has not been established, despite the high demand for such a method. Using our understanding of herpesvirus reactivation, we have developed a method for measuring the accumulation of fatigue by determining the amount of a human herpesvirus, which is reactivated and released into saliva. Our study has revealed that fatigue accumulates due to various types of stress for a period of 1 week or more can be quantitatively determined with this method.

Development of a safe and simple technique for increasing the concentration of a virus or viral vector

Various viral vectors have been developed for genetic therapies and vaccine therapies, and positive results from their use are expected. However, because desirable effects cannot be obtained when the concentration of a viral vector is low, the concentration must be increased to an adequate level. Because the success of genetic therapy depends on the efficiency of gene introduction into cells, viral vectors that can introduce genes in

a highly efficient manner are used. However, retroviral vectors, including lentiviral vectors, showing high titers have not been obtained; hence, the efficiency of gene introduction is not sufficient. Accordingly, various processes for concentrating viruses and products based on such processes have been developed, but various practical remain. We have developed a novel method for increasing the concentration of a virus or a viral vector while maintaining its infective ability and have developed a kit for it. This method is a safe and simple way to increase the concentration of a vector, such as a retroviral vector or a herpes viral vector, useful for genetic therapy, vaccines, and the like.

Molecular mechanism and major cause of fatigue

Fatigue is an indispensable biological alarm to avoid the exhaustive state caused by severe stress and overwork, which may also induce a variety of diseases. Different types of fatigue might share common mechanisms.

For years many scientists thought that lactic acid caused fatigue. However, it is now understood that lactic acid itself does not cause fatigue, because lactic acid is a key substance for providing energy and because the acidity through a build-up of lactic acid helps prevent muscle fatigue. Thus, the molecular mechanisms of fatigue remain unclear.

We have investigated the molecular mechanism of herpesvirus reactivation, which is stimulated by fatigue, and identified the molecule that can induce viral reactivation during fatigue. The molecule was up-regulated by more than 10 fold with fatigue induced by forced swimming or shortened sleep.

Our study describes a novel signal transduction pathway for fatigue and its relationship with possible fatigue-causing substances, such as cytokines, and oxidative stress.

Identification of novel HHV-6 latent protein associated with mood disorders and the molecular mechanism of fatigue due to overwork

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

We have searched for novel HHV-6 latency-associated transcripts (H6LTs) and proteins and analyzed the function and prevalence of the newly identified latency-associated protein. Then, we studied the gene regulation of H6LTs, and searched for the factor(s) that induces viral reactivation.

We identified a novel HHV-6 latent transcript that was expressed during the relatively activated latent stage (intermediate stage) of HHV-6 latency. This transcript encoded the small open reading frame of a peptide, which we named small protein encoded by the intermediate stage transcript of HHV-6 (SITH)-1. SITH-1 significantly up-regulated the intracellular calcium levels of astrocytes. Mice expressing SITH-1 showed manic behavior. Serological studies identified antibodies against SITH-1 in 71% of patients with CFS and depressive symptoms, 53% of patients with depression, 76% of patients with bipolar disorder, 2% of healthy adults.

HHV-6 reactivation during fatigue was controlled by a small upstream open reading frame regulation mechanism that was released by the kination of eukaryotic initiation factor 2 alpha, which is a stress response mechanism in primitive eukaryote yeast.

We have identified the novel HHV-6 latent protein SITH-1, which may cause mood disorders. Furthermore, we have identified the molecular mechanism of fatigue that induces HHV-6 reactivation.

In this study we have shown that SITH-1, a protein encoded by HHV-6 during the intermediate stage of latency, is associated with mood disorders in CFS, depression, and bipolar disorder. Moreover, newly identified molecular mechanisms of fatigue may be related to HHV-6 regulation and mood disorders.

Use of HHV-6 and HHV-7 as gene therapy vectors

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

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

In particular, HHV-6 and HHV-7 have attracted interest as viral vectors for gene therapy, because infections by these viruses cause only mild symptoms. Using the herpesvirus, and HHV-6 and HHV-7 in particular, as recombinant viruses and a recombinant viral vectors has certain advantages, which include low pathogenicity, the ease of introducing genes into such blood cells as the T cells and macrophages, and the ability to introduce large genes. However, producing a recombinant virus is difficult, and at present, no method is available to produce such recombinant viral vectors from HHV-6 or HHV-7. One factor that makes the recombination of HHV-6 and HHV-7 difficult is the characteristics of the HHV-6 and HHV-7 genes.

We have identified the dispensable genes of HHV-6 and HHV-7 and have reported the establishment of recombinant HHV-6 and HHV-7. The dispensable locus of HHV-6 is approximately 8.4 kbp, and that of HHV-7 is approximately 7.3 kbp; both are useful sites for inserting large genes. The exogenous nucleotide sequence may encode at least one kind of substance selected from a group consisting of a bacterial artificial chromosomes, cytokine genes, ribozymes, interference RNA, immunological co-stimulator molecules, signal transduction molecules, enzymes, and chemical attractants. Furthermore, the exogenous nucleotide sequence may be used for the gene therapy of mammals. The gene therapy may be for preventing human immunodeficiency virus (HIV) infection of a compromised cell caused by HIV or for the immunotherapy of cancer.

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Department of Bacteriology

Yoshimitsu Mizunoe, *Professor*

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General Summary

Research projects of our department have focused on 1) the mechanism of inhibition of *Staphylococcus aureus* colonization by commensal *Staphylococcus epidermidis*; 2) fibronectin-mediated colonization via fibronectin-binding proteins (FnBPs) in *S. aureus* infection; 3) molecular mechanisms of bacterial ATP secretion; 4) the isolation and characterization of biofilm detachment factor from *S. aureus*.

Research Activities

Analysis with fnb-mutant strains of FnBP functions in in vivo and in vitro infections with S. aureus

FnBPA and FnBPB are important adhesins for *S. aureus* infection. We constructed mutant strains deficient in *fnbA* or *fnbB* or both from *S. aureus* SH1000, which has intact *rsbU*, and the role of these adhesins in a mouse model of systemic infection was studied. Intravenous infection with *fnbA*- or *fnbB*-mutants or with an *fnbA*-/*B*-mutant resulted in a marked decrease in *S. aureus* pathogenicity *in vivo*. However, only FnBPA showed an indispensable role in *in vitro* experiments: internalization into epithelial cells, endothelial cells, and fibroblasts and ingestion by macrophages. Activation of nuclear factor κ B in fibroblasts and redistribution of integrin in macrophages were also induced by FnBPA. These results indicate that FnBPA is sufficient for interaction with host cells *in vitro*, whereas both FnBPA and FnBPB are needed for the progression of systemic infection *in vivo*.

S. epidermidis Esp inhibits *S. aureus* biofilm formation and nasal colonization

Commensal bacteria inhibit pathogen colonization; however, complex host-microbe and microbe-microbe interactions have so far prevented a detailed understanding of the mechanisms involved in the inhibition of colonization. Here, we show that the serine protease Esp secreted by a subset of the commensal bacterium *S. epidermidis* inhibits biofilm formation and nasal colonization by *S. aureus*, a pathogen of humans. Epidemiologic studies have demonstrated that the presence of Esp-secreting *S. epidermidis* in the nasal cavities of human volunteers correlates with the absence of *S. aureus*. Both purified Esp and Esp-secreting *S. epidermidis* inhibit biofilm formation and destroy pre-existing *S. aureus* biofilms. Furthermore, Esp increases the susceptibility of *S. aureus* in biofilms to immune-system components. Studies in human subjects have shown that Esp-secreting *S. epidermidis* eliminates *S. aureus* nasal colonization. These findings indicate that Esp hinders *S. aureus* colonization *in vivo* via a novel mechanism of bacterial interference, which could lead to the development of novel treatments to prevent *S. aureus* colonization and infection.

Isolation and identification of ATP-secreting bacteria from mice and humans

A recent report has shown that ATP causes colitis in mice via the differentiation of T helper 17 cells. Although ATP has been suggested to be secreted by commensal bacteria in the murine intestine, such ATP-secreting bacteria had not been isolated or identified. In the present study, we isolated and identified ATP-secreting bacteria from mice and humans. In humans, assessment of the relationships between the ATP-secreting bacteria and colitis will facilitate the understanding of various aspects of colitis, including the pathology, development of treatments, prophylaxis, and prognosis. Because ATP is essential for the organisms, the extracellular secretion of ATP may indicate an unknown symbiotic relationship with a pathogen or commensal bacterium in the microbial flora of the gut.

*Analysis of a biofilm detachment factor secreted by *S. aureus**

Biofilms are communities of microorganisms within a polymeric matrix that are attached to a surface. Bacterial biofilms are found on such diverse surfaces as rocks in the oceans, medical devices, and water pipes in the factory and home and cause contamination that is difficult to eliminate. The bacteria within the biofilm matrix are protected from the host immune system and from antibiotic attack. As a result, biofilm formation is a major determinant of virulence in chronic bacterial infections. Therefore, finding a substance that can disassemble biofilms might have wide-ranging medical and industrial applications to prevent or eradicate biofilms. *S. aureus* causes a wide range of acute and chronic human infections. The organism can attach to solid surfaces of native tissues or artificial devices, thereby forming a biofilm matrix that encases the bacteria. We found that *S. aureus* secretes a factor that detaches the *S. aureus* biofilm. The factor responsible for the detachment effect is a heat-stable, water-soluble molecule. When supernatants fractionated through filters of different molecular sizes were added to the biofilm, the biofilm was detached by the <1 kDa fraction. On-going studies focus on the identification of the factor.

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Department of Public Health and Environmental Medicine

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 Yuichi Miyakoshi, *Assistant Professor*

General Summary

Our major research projects in the 2009 academic year focused on: 1) genotoxic effects of indium hydroxide on micronucleus induction; 2) the analysis of 8-hydroxydeoxyguanosine by means of gas chromatography/mass spectrometry (GC/MS); 3) DNA damage by exposure to electromagnetic fields; 4) effects of zinc deficiency on the induction of chromosome aberrations; 5) oxygen-induced oxidative stress; 6) methods of medical informatics education and evidence-based medicine (EBM); and 7) the risk of decompression sickness.

Research Activities

Experimental Medicine

1. A possible mechanism for the enhancement by co-exposure to static magnetic fields (SMFs) of micronucleus formation by mutagens

We used an *in vivo* micronucleus test to investigate the effects of the superoxide scavenger 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPOL) on the co-mutagenic effect of SMFs and X-rays. The micronucleus frequency induced with X-rays was increased by co-exposure to SMFs, but this increase was inhibited by treatment with TEMPOL. Exposure to SMFs can enhance micronuclei by affecting the behavior of free radical species produced within cells.

2. Mutagenicity of electromagnetic fields and active oxygen species

Our study shows that the promotion of mutagenicity (micronuclei) in newborn rat astrocytes by exposure to electromagnetic fields is partially related to active oxygen species because the mutagenicity induced by bleomycin is increased by electromagnetic fields and is suppressed by TEMPOL, a superoxide scavenger.

3. Oxidative stress by oxygen breathing in neonates

To clarify the carcinogenicity of target organs by oxygen exposure during the neonatal period, we studied oxidative DNA damage according to 8-hydroxydeoxyguanosine (8-OHdG) in newborn rats. The oxidative DNA damage was observed which is not immature as for the oxidative defense system in the neonatal period.

4. A method for analyzing 8-OHdG with GC/MS

8-OHdG is considered the best variable for assessing oxidation damage of DNA. The measurement of 8-OHdG using GC/MS was examined.

Epidemiology, EBM, investigation, and medical informatics

1. EBM

A systematized body of epidemiologic principles with which studies can be designed and

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

2. The effect of fasting plasma glucose variability on the risk of retinopathy in patients with type 2 diabetes: Retrospective long-term follow-up

To clarify whether fasting plasma glucose (FPG) variability can be used to predict the development and progression of diabetic retinopathy independently of glycemic control, we performed a long-term follow-up study in patients with type 2 diabetes. We found that FPG variability is a risk factor for mild-to-moderate and severe nonproliferative diabetic retinopathy independent of the mean FPG or HbA1c in patients with type 2 diabetes.

3. Mental health in the workplace

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

4. Pressure ulcer healing and zinc supplementation with polaprezinc

We examined whether the healing of pressure ulcers would differ before and after treatment with the zinc-containing preparation polaprezinc in patients with chronic pressure ulcers. The healing rate of pressure ulcers was significantly higher after treatment than before. This result suggests that polaprezinc is useful for promoting the healing of pressure ulcers.

5. Questionnaire survey for professional divers

The occupational health of professional harbor divers was analyzed by means of a questionnaire survey about the operations management and prevention of dysbarism under their working conditions. There were many problems concerning recompression therapy for diver's disease based on the Industrial Safety and Health Act.

Publications

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Department of Forensic Medicine

Kimiharu Iwadate, *Professor*

Kenji Fukui, *Assistant Professor*

General Summary

Our main research projects in 2009 have focused on sudden unexpected infant death due to milk aspiration, the diagnosis of drowning by detection of specific DNA fragments of aquatic bacteria from blood samples, the analysis of death associated with medical adverse events, personal identification of war-dead remains by means of DNA analysis, the objective evaluation of the limits of DNA typing based on the intensity of ninhydrin treatment, and the quantitative analyses of medicines and poisonous substances in forensic autopsy cases.

Research Activities

Forensic pathology

1. Sudden unexpected infant death due to milk aspiration

To examine longitudinal changes in pathological findings of the lung and other organs in cases of milk aspiration, an experimental study using a murine model was performed. The results of immunostaining with an anti-human α lactalbumin antibody indicated that the kidneys and spleen, as well as the lung, showed positive reactions against the antibody over time. The detection of aspirated milk in organs other than the lung would be clear evidence of intravital milk aspiration and suggests previous or recurrent milk aspiration.

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

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

3. Analysis of death associated with medical adverse events

Public concerns about medical safety have recently increased as the Ministry of Health, Labour and Welfare have implemented associated projects. This study focused on the characteristics of deaths associated with medical adverse events and was based on autopsies performed by the department of forensic medicine. We identified cases associated with potential diagnostic errors and system errors as well as cases associated with performance errors.

DNA analysis

1. Identification of war-dead remains with DNA analysis

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

2. 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.

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 (GC), GC/mass spectrometry (MS), and spectrum photometry in samples obtained at autopsy.

2. Qualitative and quantitative analyses of hydrogen sulfide

We performed autopsies in 17 cases of fatal hydrogen sulfide poisoning due to the inhalation of intentionally generated hydrogen sulfide gas. The concentrations of sulfide and thiosulfate in blood, urine, cerebrospinal fluid, and pleural effusion were examined with GC/MS. In previous reports, the blood concentration of thiosulfate was higher than that of sulfide in cases of hydrogen sulfide poisoning, although in the present study the concentration of sulfide was higher than that of thiosulfate in 8 of the 14 cases examined.

3. Applicability of a CO oximeter to forensic autopsy cases

The applicability to forensic autopsy cases of the CO oximeter was examined. The carboxyhemoglobin (COHb) saturation was examined in cases of death by fire, of CO intoxication other than death by fire, and of other causes of death, by means of 2 types of CO oximeters and a spectrophotometer. The COHb saturation could be measured with the CO oximeters in all samples except for those with putrefaction or heat denaturation. The COHb saturation measured with the CO oximeters tended to be somewhat lower than that measured with spectrophotometry. In conclusion, the CO oximeter is useful clinically and forensically for the measurement of COHb saturation.

Radiocarbon analysis

1. Establishment of age estimation

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

Publications

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Department of Tropical Medicine

Naohiro Watanabe, *Professor*
Kenji Ishiwata, *Associate Professor*

Asao Makioka, *Associate Professor*
Masahiro Kumagai, *Assistant Professor*

General Summary

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

Research Activities

Mast cells and basophils in infection

We have performed studies to test our hypothesis that mast cells and basophils play roles in immune protection and regulation. Recently, the expression of Dectin-1, a molecule related to innate immunity, was reported on mast cells. Then, the protective role of Dectin-1 in *Plasmodium berghei* infection was examined in mice. Deletion of Dectin-1 on mast cells resulted in decreased protection, suggesting that Dectin-1 on mast cells has a protective role. In dengue virus infection in Vietnam, the sera of patients with hemorrhagic fever or shock syndrome exhibited elevated levels of vascular endothelial growth factor, which is a cytokine from mast cells, and higher amounts of chymase and tryptase, which are mast cell-specific enzymes. These results indicate that mast cell activation is involved in the pathogenesis of dengue virus infection. In tick infection in mice, basophils infiltrated at the skin site of infection. Moreover, mast cells and antibodies helped protect against ticks.

B7-DC and interleukin 4 on antigen-presenting cells in the T helper 2 immune response

The caliber and magnitude of T-cell responses are regulated by costimulatory molecules following the engagement of T-cell receptors and MHC molecules. The costimulatory molecule B7-DC has the highest homology with B7-H1 in the B7 family, and both molecules bind an immunoregulatory molecule, programmed death 1 (PD-1). Previous studies have shown that B7-DC stimulates the proliferation of T cells and the generation of cytotoxic T lymphocytes, actions that contrast sharply with the inhibitory role of B7-H1. We used an intestinal nematode, *Nippostrongylus brasiliensis*, to induce strong T helper (Th) 2 responses and to evaluate B7-DC function under Th2-polarizing conditions *in vivo*. By either blocking B7-DC expression during *N. brasiliensis* infection or by examining *N. brasiliensis*-infected B7-DC knockout mice, we observed enhanced eosinophilia, the overproduction of serum IgE, and increased Th2 cytokine production along with decreased production of Th1 cytokines, particularly interferon- γ , indicating that B7-D inhibits Th2 responses. Our results further demonstrate that the inhibition of Th2 responses by B7-DC occurs independently of PD-1 but conceivably acts through an as yet unknown alternative receptor. These results suggest that B7-DC plays an important role in bolstering a robust Th1 response, even under a strong

Th2-polarizing environment induced by *N. brasiliensis* infection. In contrast, flow cytometry and *in vitro* analysis showed that interleukin 4, a representative Th2 cytokine, reduced the antigen-presenting activity of mesenteric lymph node cells. Although several issues remain unresolved, these results suggest that regulation of signals from B7-DC and interleukin 4 ameliorate Th2-associated diseases, such as allergy and hay fever.

Expression analyses of Entamoeba chitinases in excystation and encystation

Entamoeba histolytica forms chitin-walled cysts in the infected host whose chitin must be disrupted when the amoeba excysts for infection. Chitinase, an enzyme for chitin degradation, is involved in both encystation and excystation. As *E. histolytica* does not encyst in axenic culture, we used *Entamoeba invadens*, a reptilian amoeba, as a model of *E. histolytica* because *E. invadens* can easily encyst and excyst in culture, and studied the messenger RNA expression of chitinases. We retrieved 4 chitinase homologs named EiChit1, 2, 3, and 4 from an *E. invadens* genome database. In trophozoites, Chit2 and 3 were expressed at very low levels. During encystation, the expression of all 4 chitinases increased markedly in the early phase and then decreased in the later phase. In cysts, a large amount of Chit1 and a small amount of Chit4 were expressed. During excystation, as assayed 5 hours after induction, the expression of Chit1 and 4 decreased, and that of Chit2 and 3 increased. These data demonstrate the dynamics of chitinase expression in the differentiation of *Entamoeba*.

Transcription start site and expression analyses of Entamoeba genes using an ultrafast sequencer

Transcription start site (TSS) sequencing, which is a combination of the oligo-capping method with an ultrafast sequencer, has recently been developed. This method allows us to simultaneously analyze an extensive number of TSSs and, by counting the tag numbers, the level of gene expression. Here we used TSS sequencing to investigate the TSSs of *E. invadens* trophozoites and the change in expression of genes after the induction of encystation. More than 90% of 3534 genes had a 5'-end untranslated region less than 30 base pairs in length, which was similar to data from the 'Full-Entamoeba' database previously constructed by us. As for expression in induced cysts, a chitin-binding lectin was most abundant, and chitinases were expressed at high levels, although many hypothetical proteins were also included among the top 30. Thus, the TSS sequencing was useful for simultaneous analyses of *Entamoeba* TSSs and gene expression.

Resistance against malaria in thalassemia

Persons with thalassemia have resistance to malaria. The mechanism of this resistance was examined in the thalassemia mouse but in vein. We focused on human fetal hemoglobin, which persists for the entire life in humans but not in mice. The human fetal hemoglobin gene was introduced to mice to simulate human thalassemia. This thalassemia model mouse had lower parasitemia and survives longer after infection. In addition, this mouse produced much larger amounts of fetal hemoglobin. These results

indicate that resistance to malaria in thalassemia depends on human fetal hemoglobin and not on just thalassemia.

Publications

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Department of Laboratory Medicine

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Tomokazu Matsuura, *Associate Professor*

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Kenichi Sugimoto, *Associate Professor*
Toshihiko Hashizume, *Assistant Professor*

General Summary

Research projects in our department in 2009 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

The daily intake of coffee enhances fat metabolism by altering lipolytic hormones and inhibits the development of metabolic syndrome. Therefore, a combination of coffee ingestion and moderate exercise might greatly enhance fat metabolism and decrease risk factors for metabolic syndrome. Eight healthy male volunteers ingested 250 ml of coffee containing 4 mg/kg body weight of caffeine or 250 ml of hot water as a control fluid. The present study suggests that moderate-intensity exercise after temporary ingestion of coffee inhibits glucose metabolism and preferentially enhances fat metabolism and energy metabolism during the recovery period.

Clinical microbiology

Blood stream infections and respiratory tract infections in children and adults with febrile neutropenia were studied with a magnetic filtration system for DNA/RNA extraction (Magtration, Precision System Science, Co., Ltd., Chiba, Japan), dual priming oligonucleotide polymerase chain reaction, and DNA microchip electrophoresis (MultiNA, Shimadzu Corp., Kyoto, Japan). Multiple infectious agents have been identified in both cases. Bioremediation of dioxins with thermophilic bacteria has been established for contaminated soil and has achieved reductions of 90% (W/W). Methods have been developed for treating infectious waste and chemical hazardous materials in hospitals and other institutions. Epidemiological studies were performed with the DNA diagnosis of pathogens.

Clinical chemistry

1. Genetic polymorphism of enzymes related to the therapeutic effectiveness of fluorouracil in patients with hepatocellular carcinoma

We investigated the genetic polymorphisms of 3 enzymes related to the therapeutic effectiveness of fluorouracil (5-FU) in 58 patients with hepatocellular carcinoma as follows: 1) cytochrome P450 2A6 (CYP2A6), which activates the prodrug of 5-FU

(tegafur): CYP2A6*4 (enzyme deletion type); 2) dihydropyrimidine dehydrogenase (DPD), which metabolizes and inactivates 5-FU: DPD*9AB (no activity); and 3) thymidylate synthase (TS; target enzyme): divide between TS high and low expression types by TS tandem repeat (variable number tandem repeat) and G/C polymorphism within the 28-bp repeat component of TS (single nucleotide polymorphism) (reported by Kawakami K, et al., Cancer Res 6004, 2003). Only 13 of 58 patients with hepatocellular carcinoma had normal activities of CYP2A6 and DPD and the low-expression type of TS.

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 to assess the risk of cardiovascular disease, including the application of our high-performance liquid chromatography (HPLC) method to determine lipoprotein levels.

We reported the following findings:

1. We verified the significance of measuring levels of very low density lipoprotein (VLDL) cholesterol with our established HPLC lipoprotein analysis method for monitoring the exercise-induced amelioration of lipid metabolism and the longer time required for the improvement of adiponectin and insulin resistance than for the amelioration of VLDL metabolism; a manuscript was submitted to the *Journal Atherosclerosis and Thrombosis*. The results were presented, in part, at a symposium of the 17th annual meeting of the Japan Society of Exercise and Sports Physiology.
2. A revised HPLC method for measuring lipoprotein A was developed with a collaborative project and submitted for publication in the *Journal of Lipid Research*.
3. Because of the advantages of our revised HPLC method, Hiroshi Yoshida was awarded the Life Science Prize at the 56th National Congress of the Japanese Society of Laboratory Medicine.
4. The clinical features of malondialdehyde-modified LDL, a novel method of oxidized LDL measurement, and the clinical significance of oxidized lipoproteins were presented at a symposium of the 56th national congress of the Japanese Society of Laboratory Medicine
5. Atherosclerosis-related serum lipid markers (remnant lipoprotein cholesterol and small dense LDL) was presented at a symposium of the 41st annual meeting of the Japan Society of Clinical Laboratory Automation, and our findings concerning cholesterol and atherosclerosis were presented at a seminar of the 49th annual meeting of the Japan Society of Clinical Chemistry.
6. The ameliorating effects of astaxanthin on triglyceride, high-density lipoprotein cholesterol, and adiponectin were verified and presented at a workshop of the 15th congress of the International Atherosclerosis Society (Boston, MA, USA) and at a symposium of the 7th annual meeting of the Japanese Society for Medical Use of Functional Foods and was submitted for publication in the journal *Atherosclerosis*.
7. Subanalysis by sex from the findings of the Japanese Investigation of Kinetic Evaluation in Hypertensive Event And Remodeling Treatment (JIKEI HEART) study was presented at the annual congress of the European Society of Cardiology (Barcelona, Spain) and was submitted for publication in the *Journal of Hypertension*.

Hematology

Evaluation of the QuantiFERON TB-2G test for detecting tuberculosis infection
We evaluated the efficacy of the QuantiFERON TB-2G (QFT) test (Cellestis Limited, Melbourne, Australia) in detecting tuberculosis infection by comparing the results obtained with QFT, liquid culture, the polymerase chain reaction, and direct staining. The QFT test was found to be an easy-to-use method, but some differences were found. Positive results on the QFT test were obtained in 20% of patients. The detection rate of tuberculosis with the QFT test was sufficiently high in patients younger than 50 years, but the detection rate was lower in older patients. These results indicate that the results of the QFT test depend on the history of tuberculosis infection and that the QFT test should be accompanied by bacterial tests.

Cardiology

This year we researched the following 3 topics.

- 1) As participants in a multicenter study, we analyzed the effect on health of longtime exercise with respect to oxidant stress.
- 2) From the standpoint of perioperative cardiovascular evaluation for noncardiac surgery, we studied the significance of electrocardiography as a preoperative examination.
- 3) We evaluated the effectiveness of catheter ablation for atrial fibrillation and our improvement of this method.

Clinical cell biology

1. ^{13}C -glucose breath test

The ^{13}C -glucose breath test was established as a diagnostic test for insulin resistance and as a liver function test.

2. Enzyme-linked immunosorbent assay for measurement of latency-associated protein of transforming growth factor β degradates in plasma

We have developed a novel, quantitative, and specific assay for plasma latency-associated protein of transforming growth factor (TGF) β degradates (LAP-D), which are produced during proteolytic activation of TGF- β . We have previously validated this assay as a marker of hepatic stellate cell activation in *in vivo* liver fibrosis. Plasma levels of LAP-D significantly decreased in 24 patients 3 months after the start of successful combination therapy for hepatitis C virus infection.

3. Plasma protein production system using a bioartificial liver

We cultured cells producing higher levels of human plasma protein in the radial-flow bioreactor for development of plasma protein production for medical care and reviewed culture conditions.

4. Retinoid metabolism and liver fibrosis

The cellular distribution of lecithin: retinol acyltransferase and cellular retinol-binding protein I was examined in biopsy specimens from patients with chronic viral hepatitis. Ten percent to 20% of double-positive cells were distributed in fibrous lesions in pathological livers. The results suggest that these cells are still able to store vitamin A even if they transform into myofibroblasts.

5. Diagnosis and therapy for lethal encephalopathy in acute hepatic failure
Toxin receptor-mediated cell knockout mice were used as an experimental model of lethal hepatic encephalopathy in acute hepatic failure.

6. Ultrasound molecular imaging system

We performed research to develop stable nanobubbles for use in contrast media. Our research into the application of nanobubbles to a high-sensitivity ultrasound molecular imaging system was advanced through the use of harmonic imaging.

Clinical psychiatry

Psychotropic drugs have caused some concern in clinical practice because of their ability to reduce seizure thresholds and provoke seizures. Therefore, we examined the therapeutic attitudes regarding several forms of psychoses associated with epilepsy.

Because little attention has been paid in the past, we reported the characteristics of patients with epilepsy who demonstrated social withdrawal. Moreover, we reported a case of symptomatic generalized epilepsy in which long-term control was achieved by the addition of zonisamide therapy.

Publications

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Department of Internal Medicine

Division of Gastroenterology and Hepatology

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 Yoshio Aizawa, *Professor*
 Hisato Nakajima, *Associate Professor*
 Tomohisa Ishikawa, *Assistant Professor*
 Mika Matsuoka, *Assistant Professor*
 Yasuyuki Searashi, *Assistant Professor*
 Yoshinari Miyagawa, *Assistant Professor*

Research Activities

Alimentary tract

1. The Toll-like receptor (TLR) 8-mediated activation of human monocytes was shown to inhibit expression of tumor necrosis factor-like ligand 1A (TL1A), which is believed to be a key mediator of Crohn's disease. Immune complex-induced TL1A expression in monocytes was potently inhibited by a TLR8 or TLR7/8 ligand. Furthermore, TLR8 ligands inhibited TL1A production, resulting in the almost complete inhibition of interferon-gamma production by CD4(+) T cells. Our data suggest that TLR8 activation is an important, novel pathway for the targeted treatment of Crohn's disease. Diet therapy involving the use of an n-3 polyunsaturated fatty acid food exchange table altered the fatty acid composition of cell membranes and affected the clinical activity in patients with inflammatory bowel disease. Several types of enterobacteria enhanced secretion of corticotropin-releasing hormone in dendritic cells.
2. We analyzed the correlation between the symptomatic improvement of reflux esophagitis and quality of life (QOL) in patients with gastroesophageal reflux disease (GERD) by means of the Frequency Scale for Symptoms of GERD (FSSG). The QOL decreased as the symptoms worsened. In addition, the improvement in QOL after treatment with a proton-pump inhibitor was greater among subjects with more severe dysmotility symptoms. However, no correlation was observed between symptom improvement and the rate of QOL improvement. We concluded that the efficacy of treatment with a proton-pump inhibitor was associated with the severity of symptoms before treatment.
3. Predictive factors were identified and therapeutic effects were assessed among patients with GERD by means of the FSSG. Overall, 346 (25.9%) of the 1,335 subjects who underwent health checkups were found to have GERD according to the FSSG. The sensitivity and specificity of the FSSG were 34.7% and 72.7%, respectively. A young age, excessive alcohol intake, and liver dysfunction were possible predictive factors for GERD. The FSSG score was significantly decreased by treatment with rabeprazole (20 mg \times 4 weeks+10 mg \times 8 weeks). The FSSG was useful for detecting and assessing therapeutic effects in GERD.
4. A clinicopathologic study was performed to examine risk factors, particularly

vascular invasion, for metastasis of superficial esophageal cancers to lymph nodes. We carefully analyzed the risk factors of lymph node metastasis before surgical treatment in patients with esophageal superficial cancer. We examined 110 surgically resected lesions and detected metastasis to lymph nodes in 37 cases (33.6%). We evaluated vascular invasion using special staining procedures (D2-40, elastica-van Gieson, CD31, and CD34) and statistically analyzed these and other risk factors for lymph node metastasis; vascular invasion evaluation using special staining procedures was the strongest risk factor for lymph node metastatic. The negative predictive value was 94.6%.

Liver and Pancreas

1. The clinical background and histological findings of patients with autoimmune hepatitis (AIH) were examined. Liver biopsy was useful for establishing a definitive diagnosis and evaluating treatment in patients with AIH. The blood sampling data were normalized with immunosuppressive drugs in more than 95% of the cases of AIH. A discrepancy between the clinical data and the histological activity was confirmed for some cases. The liver biopsy findings reflected the actual clinical course.
2. The relationship between the severity of sleep apnea syndrome (SAS) and liver damage was studied. We surveyed metabolic imbalances in patients with SAS. Twenty percent of patients with SAS had complications, such as obesity, dyslipidemia, and glucose intolerance. A relationship between the severity of SAS and liver metabolic imbalance under conditions of severe long-term hypoxia was suggested. Nonalcoholic fatty liver disease and SAS were thought to be associated with common metabolic imbalances.
3. Natural killer T (NKT) cells and dendritic cells in primary immunoreactions in a mouse model of AIH were analyzed. The results suggested that NKT and dendritic cells are involved in the pathogenesis of AIH. The number of intrahepatic activated NKT cells that secrete interferon- γ increased during the inflammation phase. The expression of CD1d, which activates NKT cells, on intrahepatic Kupffer cells and dendritic cells also increased during the inflammation phase. These results indicate that activated NKT cells play a significant role in this AIH model.
4. The molecular mechanism of liver stem cells in fetal liver development was studied. The marker factor Sall4 was confirmed to regulate cell fate decisions in fetal hepatic stem/progenitor cells. This molecule may have novel clinical applications. Our results suggest that Sall4 plays a crucial role in controlling the lineage commitment of hepatoblasts, not only inhibiting their differentiation into hepatocytes, but also driving their differentiation toward cholangiocytes.
5. "Validation of a Food Frequency Questionnaire Based on Food Groups and Indirect Calorimeters" was a useful method for estimating the nutritional status of individual patients. We studied imbalances in the nutritional status of patients with chronic liver disease. Sixty-five percent of patients with liver cirrhosis exhibited excessive protein consumption. Thus, assessment of nutritional status was considered necessary for clinical care.
6. The relation between connective tissue growth factor (CTGF) and liver fibrosis was

studied. Consequently, CTGF was shown to be a peptide marker of fibrosis and angiogenesis. We measured plasma CTGF levels in patients with chronic liver disease. The plasma CTGF levels were higher in patients with liver cirrhosis than in patients with chronic hepatitis. A comparison of the plasma CTGF level and the pathological stage of liver fibrosis showed a good agreement. In addition, stellate cells positive for both lecithin:retinol acyltransferase and cellular retinol-binding protein 1 contributed to portal fibrosis in human liver disease.

7. The specific characteristics of liver dysfunction were examined at an annual company health checkup. We studied physical measurements, insulin resistance, and the characteristic liver damage pattern for the early identification of nonalcoholic fatty liver disease. Obese participants were found to have large liver fat stores and a high rate of liver dysfunction.

8. The effectiveness of magnetic resonance (MR) for visualizing hepatocellular carcinoma (HCC) was examined. Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) is a new enhancement medium for the detection of HCC using MR. Detailed MR images obtained using Gd-EOB-DTPA and the clinical stage of HCC were analyzed. About 80% of the enhanced MR findings obtained with Gd-EOB-DTPA agreed with those obtained using hepatic artery angiography. This noninvasive enhanced MR technique was thus considered an effective imaging method.

9. A ^{13}C -glucose breath test was established as a diagnostic test for insulin resistance and as a test of liver function.

10. We have developed a novel, quantitative, and specific assay for the degradation products of plasma latency-associated protein of transforming growth factor (TGF) β (LAP-D), which are produced during proteolytic TGF- β activation. This assay has previously been validated as an in vivo marker of hepatic stellate cell activation during liver fibrosis. The plasma levels of LAP-D were significantly decreased in 24 cases 3 months after the start of successful combination therapy for hepatitis C virus (HCV) infection.

11. The relationship between regulatory T cells in the peripheral blood of patients with HCV infection and the progression of liver disease, including HCC, was studied. The apolipoprotein B level was found to be associated with the virological response in patients receiving treatment for chronic HCV infection.

12. Vaccination with Wilm's tumor protein 1 in combination with gemcitabine was examined for the treatment of patients with advanced pancreatic cancer. This therapy may be effective for patients with advanced pancreatic cancer.

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Department of Internal Medicine

Division of Neurology

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General Summary

Our research in 2009 was conducted in the following areas: 1) sexual dysfunction in Parkinson disease (PD), 2) autonomic dysfunction in neurodegenerative disease, 3) neurophysiological studies of the visual information processing functions and diabetic polyneuropathy, 4) neuroradiological studies with nuclear medicine, 5) ultrasonological studies of cerebrovascular disease, and 6) basic research in motor neuron disease and in the axonal plasticity of the central nervous system.

Research Activities

Sexual dysfunction in PD

Sexual dysfunction often occurs in PD. In this study, we used the Beck Depression Inventory, Second Edition, to evaluate depression. The relations of the degree of sexual debility to depression and autonomic disorders were studied. We concluded that the degree of sexual debility in PD is associated with the severities of depression and autonomic disorders.

Autonomic dysfunction in neurodegenerative disease

We studied the cardiovascular autonomic dysfunction in patients with Lewy body disease, such as PD or dementia with Lewy bodies. The autonomic function was evaluated using cardiac metaiodobenzylguanidine (MIBG) scintigraphy, hemodynamic function test by Valsalva maneuver and orthostatic tolerance test. Using these methods, we estimated the characteristics of subclinical autonomic nervous dysfunction in de novo PD without orthostatic hypotension (OH). We also studied the relation of olfactory dysfunction to cardiovascular dysautonomia in patients with PD. Olfactory dysfunction in PD was thus significantly related to both cardiac sympathetic and parasympathetic dysfunction, as well as vascular sympathetic dysfunction. As nonmotor symptoms of PD, olfactory dysfunction and autonomic network failure appear to be closely related in PD.

Cliniconeuropathological evaluation of the olfactory bulb in PD

We investigated the incidence and extent of Lewy body-related alpha-synucleinopathy (LBAS) in the olfactory bulb in 320 consecutive patients examined at autopsy. Paraffin-embedded sections were immunostained with antibodies against phosphorylated alpha-synuclein, tyrosine hydroxylase, phosphorylated tau, and amyloid- β . LBAS was

found in the central nervous system of 102 patients and in the olfactory bulb of 85 patients. All 35 patients who showed LBAS with pigmentation loss in the substantia nigra had LBAS in the olfactory bulb. LBAS in the amygdala was more strongly correlated with LBAS in the anterior olfactory nucleus than with that in the periphery of the olfactory bulb. These results indicate a high incidence of LBAS in the aging human olfactory bulb; they also suggest that LBAS extends from the periphery to the anterior olfactory nucleus and results in the clinical manifestations of Lewy body disease.

Neurophysiological studies of the visual information processing functions and diabetic polyneuropathy

Visual information processing functions were compared by means of visual and auditory event-related potentials in patients with PD, dementia with Lewy bodies, and Alzheimer's disease (AD).

The clinical utility of nerve conduction studies and neurological examination of the feet with newly established techniques was assessed, in collaboration with the Department of Diabetes, Metabolism and Endocrinology, in patients with diabetes mellitus but no sensory symptoms in the feet. The findings of the study suggest that 34% of patients with diabetes mellitus have subclinical polyneuropathy.

Neuroradiological studies with nuclear medicine

Brain perfusion images were compared using statistical imaging methods, such as 3-dimensional stereotactic surface projection of ^{123}I -isopropylidoamphetamine single-photon emission computed tomography (SPECT) and easy Z-score imaging system of $^{99\text{m}}\text{Tc}$ -ethylcysteinate dimer SPECT, among patients with dementing and parkinsonian disorders. These novel methods demonstrated the spectrum of pathological involvement of the cholinergic and dopaminergic projections of AD and PD, suggesting their usefulness in routine clinical practice.

Amyloid imaging has recently emerged as a promising tool that enables us to evaluate the progression of disease objectively by assessing in vivo accumulation of amyloid- β in the brains of patients with AD. The purpose of this study was to directly compare the characteristics of 2 amyloid probes, [^{11}C] Pittsburgh Compound-B (PIB) and [^{11}C] BF-227, in the same patients with AD. The sensitivity of [^{11}C] PIB for detecting amyloid- β accumulation may be much higher than that of [^{11}C] BF-227. However, the difference in the distribution of the 2 probes presumably reflects the difference in the specificity to amyloid- β , or the difference in the affinity to the different stage of amyloid- β aggregation in the senile plaque generation process, or both. Therefore, these differences may provide additional pathophysiological information about amyloid cascade in AD.

Ultrasonological studies of cerebrovascular disease

Cerebrovascular ultrasonography is useful for evaluating cerebral hemodynamics rapidly and in real time for patients with acute ischemic stroke. We evaluated the occlusion of intracranial arteries using transtemporal and suboccipital window by transcranial color flow imaging with thrombolysis in brain ischemia flow-grading system and monitored

residual flow in real time every 15 minutes until 120 minutes after a bolus injection of tissue plasminogen activator (tPA). We monitored complete recanalization within 60 minutes after bolus injection of tPA in 2 of the 3 patients who had a good echo window. We conclude that real-time ultrasound monitoring is useful for evaluating the thrombolytic effect of t-PA.

Physiological role of monocarboxylate transport in the maintenance of hypoglossal motor neuron activity

In hypoglossal motor neurons, monocarboxylate transported by monocarboxylic acid transporter does not play a major role in maintaining the resting potential, which is likely to be maintained by ATP of other origins and makes a major contribution to the maintenance of excitatory synaptic transmission. The energy from monocarboxylate is mainly consumed at synapses.

Assessment of functional recovery and axonal plasticity in paired immunoglobulin-like receptor B-deficient mice after traumatic brain injury

The myelin-associated proteins Nogo, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein inhibit axonal plasticity. Each protein interacts with both the Nogo receptor and paired immunoglobulin-like receptor B (PirB). We examined whether blocking PirB activity enhances axonal reorganization and functional recovery after cortical injury. However, our results suggest that blocking the function of PirB is not sufficient to promote axonal reorganization or functional recovery after cortical injury.

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Department of Internal Medicine

Division of Kidney and Hypertension

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General Summary

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

Research Activities

Nephrology

1. Glomerulonephritis

Nephron numbers have recently been reported to be involved in the development of hypertension and chronic kidney disease. Our study suggested that an imbalance in individual body mass index and glomerular number is an independent risk factor for the progression of chronic kidney disease.

In *Nephrin-Bmp4* transgenic mice, the development of glomerular capillary tuft was markedly impaired, due to the lack of endothelial and mesangial cells. In contrast, *Nephrin-Noggin* transgenic mice exhibited collapsed glomerular capillary tufts due to a lack of mesangial cells.

We have established the transgenic estrogen receptor (ER)-E2F1 suicide-inducible mouse, which expresses ER-E2F1 fusion protein. E2F1 is a transcription factor that regulates cell proliferation; its ectopic expression induces apoptosis in differentiated cells. Therefore, cells of the ER-E2F1 mouse can be eliminated on demand by administering tamoxifen.

2. Dialysis

We evaluated the clinical value of the combination of peritoneal dialysis and hemodialysis. We found that this combined therapy is a useful way to control body fluids and that peritoneal function may be maintained with long-term treatment.

We studied acute humoral rejection and attempted to perform ABO-incompatible renal transplantation and husband-to-wife renal transplantation. In transplant glomerulopathy, glomerular expression of plasmalemmal vesicle-associated protein 1 is positively correlated with the severity of transplant glomerulopathy and proteinuria.

In clinical studies, we evaluated the role of collagen enzymatic and glycation-induced cross-links as a determinant of bone quality in patients with secondary hyperparathyroidism.

Hypertension

A study of the awareness of home blood pressure measurement among physicians who deal with hypertension was a major research project in 2009. This study is unusual in that it focuses on home-based blood pressure measurement. Because the guidelines released by the Japanese Society Hypertension in 2008 are the only guidelines to consider home blood pressure measurement, highlighting the importance of such measurement is important.

Uric acid metabolism

Sevelamer decreased serum urate levels, possibly by adsorbing urate in gastrointestinal tract in patients undergoing maintenance hemodialysis.

Fenofibrate decreased serum uric acid levels by increasing its urinary excretion, most likely through the inhibition of urate transporter 1 (URAT1) by fenofibric acid, its major metabolite, in renal proximal tubules.

An investigation of uric acid dynamics and staining of URAT1 after renal transplantation showed that serum uric acid levels were related, in part, to the expression of URAT1 in the proximal renal tubule.

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Department of Internal Medicine

Division of Rheumatology

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General Summary

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

Research Activities

Clinical and experimental studies of autoimmune disease were performed.

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

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

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

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

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

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

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

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

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Department of Internal Medicine

Division of Cardiology

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General Summary and Research Activities

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

Clinical research

In clinical research, we have been vigorously participating in multicenter collaborative studies, including large-scale clinical studies, and conducting research during routine clinical practice. In the large-scale clinical studies, we have primarily collaborated in subanalyses of the Japanese Investigation of Kinetic Evaluation in Hypertensive Event And Remodeling Treatment (JIKEI HEART) Study, whose results were previously reported, and in such studies as the Japanese Rhythm Management Trial for Atrial Fibrillation (J-RHYTHM) II (upstream drug therapy for atrial fibrillation associated with hypertension by means of a multicenter study: a study related to and comparative study of calcium antagonists and angiotensin receptor blocker: ARB), the Assessment of β -Blocker Treatment in Japanese Patients with Chronic Heart Failure (J-CHF) study (a large-scale clinical study to establish a β -blocker treatment method in chronic heart failure), the PEARL study (multicenter cooperative study to investigate the ameliorative effect of hydroxymethyl glutaryl coenzyme A reductase inhibitors on chronic heart failure), and the Nationwide Gender-specific Atherosclerosis Determinants Estimation and Ischemic Cardiovascular Disease Prospective Cohort (NADESICO) study (multicenter cooperative prospective cohort study on sex differences in risk factors for arteriosclerotic diseases and prevention), which used computed tomography examinations of coronary arteries.

We have converted patient data, including risk factors, lesion morphology, and other variables obtained during catheter examinations and treatment in various clinical research divisions into a database and compared risk factors, outcomes, and other clinical variables of ischemic heart disease, cardiomyopathy, and other conditions. In addition, we have participated in nationwide clinical studies (The Japan-Drug Eluting Stents Evaluation; a Randomized Trial [J-DEsSERT], the Coronary Spasm Association [CSA]), mainly investigating treatment with drug-eluting stents and the diagnosis of coronary vasospasm, which is closely related to the etiology of ischemic heart disease. In regard to heart failure, which is an extremely common circulatory disease, we have

been assessing data related to serum brain natriuretic peptide concentrations, which are an index of pathologic change, and have been performing research on standard values that will be useful in clinical practice. In addition, we have described in detail the pathologic changes of heart failure before and after admission to the hospital and are assessing clinical data that will serve as new indexes.

We have been aggressively treating atrial fibrillation by means of catheter ablation, and during this fiscal year we have treated approximately 160 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

Our research activities include studies at other institutions in Japan and abroad by graduate students in basic sciences and clinical sciences and the presentation of study results. In the field of arrhythmia, we have studied the development of atrial fibrillation by using various experimental models to examine 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 with molecular biology techniques and physiologic techniques. Other research topics include a new signal transmission system in the α -receptor stimulation effect in relation to L-type Ca channels in the rat myocardium, the effects of β -receptor stimulation in sarcoplasmic reticulum function, and cardiomyocyte intracellular Ca kinetics in mice with dilated cardiomyopathy due to troponin T mutations. In the field of myocardial metabolism, we have investigated the association between ischemia-reperfusion damage and intracellular ion kinetics in the isolated perfused hearts of mice with type 2 diabetes.

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General Summary

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

Research Activities

Epidemiology and evidence-based medicine

A nationwide epidemiologic study of mortality in approximately 3,500 patients with type 1 diabetes was started in 1986 and has continued to provide much information about the prognosis of Japanese children with type 1 diabetes. A population-based interventional study of childhood obesity and glucose intolerance has also continued. Several clinical trials of the treatment of type 2 diabetes using continuous glucose monitoring are being performed.

Molecular diabetology and islet medicine

Injuries to islet cells and the reduced regenerative capacity are novel therapeutic targets in the pathophysiology of diabetes. Our study group has succeeded in direct in vivo transfer of genes, including cell-cycle regulators. We confirmed that regulated proliferation of mature beta cells restored glucose metabolism in diabetic mice. This year, we have also begun to focus on peri-islet Schwann cells to investigate their critical role in the protection of islet endocrine cells. We found by a developmental engineering technique in mice that peri-islet Schwann cells are derived from the neural crest.

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

Dietary therapy

A highly monounsaturated enteral formula suppressed postprandial hyperglycemia,

without exaggerated insulin secretion, to a greater extent than did a high-carbohydrate enteral formula in patients with type 2 diabetes mellitus and in healthy subjects. Continuous glucose monitoring showed that a highly monounsaturated enteral formula significantly suppressed postprandial hyperglycemia and markedly reduced the 24-hour glycemic variations in tube-fed patients with type 2 diabetes to a greater extent than did a high-carbohydrate enteral formula, even if carbohydrate nutrients were adjusted to have a low glycemic index.

Diabetic vascular complications

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

Endocrinology

To identify and separate stem-like cells in human pituitary adenomas, we focused on the expression of CD133, a tumor stem-cell marker in brain tumors, and examined the differences indicating stem properties between CD133(+) cells and CD133(−) cells.

The effects of Ca-channel antagonists on the expression of steroidogenic enzymes were evaluated with a human adrenocortical carcinoma cell line, NCI-H295R.

The 12-lipoxygenase (12-LO) pathway may be involved in the pathogenesis of diabetic cardiomyopathy. Therefore, the role of 12-LO in cardiomyopathy was studied in vivo in a rat model of diabetic cardiomyopathy and in vitro with a primary cardiomyocyte culture system.

The possible direct effect of endotoxin on human stellate cells, which play a critical role in the progression of nonalcoholic steatohepatitis, was studied using LX-2 human stellate cells.

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

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Department of Internal Medicine

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General Summary

The immediate goals of our clinical and basic research are to investigate basic and clinical aspects of malignant diseases and to improve outcomes for patients with hematological malignancies and solid tumors, leading to the ultimate goals of improving the natural history of malignant diseases. We have also been performing several clinical trials and basic research studies throughout 2009.

Research Activities

Leukemias

Many patients with previously untreated hematological disorder have been referred to our department. The disorders in 2009 included acute myeloid leukemia (AML), 8 cases; acute lymphoblastic leukemia (ALL), 6 cases, chronic myeloid leukemia (CML), 2 cases; and myelodysplastic syndrome (MDS), 7 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 2009 were as follows: AML/MDS-HR CS-7 study of newly diagnosed AML, refractory anemia with excess blasts II, all-case registration cohort study; AML-201 VLA4 study (prognosis evaluation study); AML-206 DNR assigned group (relapse and refractory AML: phase I); APL-204 (phase III); APL-205R (relapsed and refractory APL: phase II); and ALL-202 (phase III). We also performed several collaborative group studies and pilot studies: Aged Double-7 (newly diagnosed aged AML: phase II); VEGA (MDS: phase II); nilotinib (refractory CML: phase I/II); and dasatinib study (refractory CML: phase I/II).

Lymphomas

In 2009 we registered 48 patients with newly diagnosed non-Hodgkin's lymphoma (NHL) and 4 patients with Hodgkin's lymphoma (HL). We have performed clinical trials as a member of the Lymphoma Study Group of the Japan Clinical Oncology Group (JCOG-LSG). Important protocol studies in 2009 were JCOG0211-DI (newly diagnosed natural killer/T lymphoma: phase I/II) and JCOG0203-MF (newly

diagnosed follicular lymphoma: phase III). Other cooperative studies examined biweekly rituximab, etoposide, prednisone, vincristine, hydroxydaunorubicin (R-EPOCH: relapsed and refractory B cell lymphoma: phase II); pirarubicin, cyclophosphamide, vincristine, and prednisolone (THP-COP: newly diagnosed T-cell lymphoma: phase II); and enzastaurin (NHL: phase III double-blind).

Enzastaurin is a novel drug targeting protein kinase $C\beta$ that has been extensively studied throughout the world, including the United States, the European Union, and Japan.

Myeloma

We registered 8 patients with newly diagnosed multiple myeloma (MM) in 2009. Bortezomib, a novel proteasome inhibitor, became available in 2007, and we have used it with or without dexamethasone to treat patients who have refractory myeloma.

Hematopoietic stem cell transplantation

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

Solid tumors

Many patients with solid cancer have been referred to our department from related divisions or departments in our hospital and outside our hospital. Several of our studies are in progress throughout our university hospital with related divisions or departments, seeking improved therapeutic outcomes. Fluorouracil (5-FU), epirubicin, and cyclophosphamide (FEC100) with or without taxotere therapy is an adjuvant therapy for patients with breast cancer treated with curative surgery. FEC100 followed by taxotere is a preoperative combination chemotherapy for patients with locally advanced breast cancer. Adriamycin and taxotere followed by taxotere and trastuzumab is a first-line chemotherapy for patients with advanced metastatic breast cancer. The standard treatment for operable, locally advanced esophageal cancer has been altered, resulting in the use of chemoradiation therapy rather than surgical resection alone or the combination of resection and radiotherapy. We, therefore, 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 such patients since late 2008. For patients with advanced gastric cancer, a combination chemotherapy of S-1 and cisplatin has been performed. Our first-line chemotherapies for patients with advanced colorectal cancer are folinic acid, 5-FU, and oxaliplatin (FOLFOX) and folinic acid, 5-FU, and irinotecan (FOLFIRI). Because antibodies against vascular endothelial growth factor and epidermal growth factor receptor became available in 2007 and 2008, respectively, combination therapies with these antibodies and FOLFOX or FOLFIRI were also performed.

Palliative care

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

Basic research

One of our important activities is translational research on hematological malignancies and solid cancers. The structural differences between M protein produced by myeloma cells and that from monoclonal gammopathy of undetermined significance have been examined, and the function of ATP-binding cassette transporters in cancer chemotherapy has been studied in collaboration with the Keio University Department of Pharmacy. We examined the conditions required for home death analysis of 9 outpatients who received chemotherapy.

Publications

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General Summary

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

Research Activities

COPD

Clinical research concerning the incidence of COPD in patients with diabetes mellitus, coronary artery diseases, or heart failure was developed. Clinical research was started concerning the effect on comorbidities of intervention for COPD, cardiovascular diseases, or diabetes mellitus. Serum levels of proinflammatory cytokines, such as tumor necrosis factor, interleukin (IL) 1, and IL-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 speculate that early intervention against COPD may prevent various comorbidities.

Acute lung injury

Double-stranded RNA viruses are associated with acute lung injury. We investigated the effect of insulin on epithelial cell fate after damage by polyinosinic:polycytidylic acid (poly IC). We used human bronchial epithelial primary culture cells and found that insulin was required to protect these cells from apoptosis induced by poly IC. Apoptotic signals were dependent on caspase-8 activation. We also found that survival signals occurred mainly through the activation of extracellular signal-regulated kinase and Akt, although other survival signals may also be associated. We suggest that insulin is a promising agent against acute lung injury induced by viral infection.

Idiopathic pulmonary fibrosis

Aberrant re-epithelialization with bronchial epithelial cells is a prominent pathologic finding in idiopathic pulmonary fibrosis (IPF) and is implicated in abnormal epithelial-mesenchymal interactions. Recent studies show that senescence is a risk factor for the development of IPF. Among the sirtuin (SIRT) family of proteins, which are class III histone deacetylases (HDACs), SIRT6 has been demonstrated to antagonize senescence. We examined epithelial senescence as a representative phenotypic alteration in conjunction with SIRT6 expression in IPF. We have produced evidence that lungs with IPF show enhanced senescence and a concomitant increase in SIRT6 expression in epithelial cells, including aberrantly re-epithelialized bronchial cells. Transforming growth factor (TGF) β induces senescence by increasing p21 expression and induces SIRT6 expression, and artificial overexpression of SIRT6 efficiently inhibits TGF- β induced senescence via proteasomal degradation of p21 in human bronchial epithelial cells. Secretion of IL- β 1 from epithelial cells induced by TGF- β to undergo senescence is responsible for myofibroblast differentiation in fibroblasts. These findings shed light on the accelerated epithelial senescence in the pathogenesis of IPF and suggest a possible regulatory role for SIRT6.

Autophagy in bronchiolar epithelial cells

To investigate the significance of autophagy in lung diseases, we examined the association between autophagy and senescence in bronchial epithelial cells. Cigarette smoke extract induced the senescence of bronchial epithelial cells. Cigarette smoke extract transiently up-regulated autophagy but then down-regulated autophagy in these cells. Cigarette smoke extract increased misfolded proteins and ubiquitinated proteins and induced the senescence of these cells. Autophagy digested these unnecessary proteins and protected these cells from senescence. We suggest that autophagy plays important roles in maintaining homeostasis in lung epithelial cells.

Pulmonary infection

An investigation of biomarkers for infectious lung diseases was started. This study focused on the significance of procalcitonin in the diagnosis and treatment of pulmonary infection.

Lung cancer

Clinical research into the effects of nitroglycerin on chemotherapy in non-small cell lung is ongoing. 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

Bronchial asthma

Clinical research concerning the step-down of inhalation treatment against bronchial asthma has been started. This study is a prospective, randomized controlled study.

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Research Activities

Division of General Medicine, The Jikei University Hospital

The objective of this study was to examine the use and effects of medical health care information among patients who had first visited our outpatient clinic. We used a questionnaire created for this cross-sectional study.

About 40% of the outpatients visited our clinic with medical health care information. Half of the information came from the Internet or from acquaintances. Little information came from newspapers or TV programs. All study participants who used the Internet had obtained information about diseases and symptoms, and half of them had obtained information about medical institutions. All study participants who had information from acquaintances had obtained information about diseases, symptoms, and medical institutions. These tendencies did not differ significantly with sex or age.

Division of General Medicine, The Jikei University Aoto Hospital

The effects of *shinrin-yoku* (forest-air bathing and walking) were investigated in patients with essential hypertension. Basic research on heart failure was performed in collaboration with a group from Essen University in Germany.

Division of General Medicine, The Jikei University Daisan Hospital

1. Study of factors of infection in elderly inpatients

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

2. Study of fever of unknown origin

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

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 achievement in rural areas, especially in Kashiwa City, and the second is to evaluate lipid abnormalities that result in premature atherosclerosis and to determine therapeutic measures for atherosclerotic disorders.

1. Investigation of the role of general medicine on environmental health achievement

We developed a new regional nutritional education system for subjects with diabetes mellitus or metabolic syndrome in the Kashiwa area using grants from the Kao Research Council for the Study of Healthcare Science in 2008 and from the Chiba Foundation for Health Promotion & Disease Prevention in 2010. As a committee member of local governance, we also helped develop a local health care system in the Kashiwa area.

2. Studies of lipid metabolism and atherosclerosis

1) We studied the effects of carbohydrate co-feeding on postprandial hyperlipidemia by measuring serum levels of apolipoprotein B-48.

2) We performed an incubation study using bacteriophages to examine the antiviral effects of plasma fractions.

3) With our newly developed method of high-performance liquid chromatography (reported in *Clinical Biochemistry* in 2007 and in *Lipids in Health and Disease* in 2008), we established a method for the measurement of lipoprotein A (reported in the *Journal of Lipid Research* in 2009).

4) The clinical significance of malondialdehyde-modified low-density lipoprotein, a clinical measurement of serum oxidized low-density lipoprotein, was assessed.

5) Effects of astaxanthin on triglyceride, high-density lipoprotein, and adiponectin were investigated, and the results were reported at the annual scientific meeting of the Japanese Society of Clinical Nutrition.

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General Summary

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

Research Activities

Psychopathology, psychotherapy and child study group

Studies examined: 1) the care systems for developmental disorders (attention-deficit hyperactivity disorder and pervasive developmental disorder) in the psychiatry unit; 2) the psychopathology underlying acute psychosis observed in some developmental disorders, especially the relationship between age and changes in scores of the Wechsler Intelligence Scale for Children-II; 3) diary therapy for Asperger disorders. A study of ground factors of depression in white-collar workers found close relationships between depression and personal characteristics, feelings of self-esteem, and psychological stress experienced outside, rather than inside the workplace.

Morita therapy group

“Guideline for Outpatient Morita therapy” was translated into English and presented at the 7th International Congress of Morita Therapy. A study of the character profiles of patients with chronic depression was completed. In this study, low scores for “Openness” and “Agreeableness” of the revised NEO Personality Inventory were found to be characteristics of patients with chronic-depression. Subjects of studies continued this year included: the relationship between panic disorders and generalized anxiety disorders from the aspect of patients’ character and comorbidity, subtypes of obsessive-compulsive disorder, “fall-in reaction” occurring in the development of mood or anxiety disorders, and factors in the recovery of patients with depression treated with inpatient Morita therapy.

Psychopharmacology group

In basic research, 1) the mechanism of central action of a new generation of psychotropic drugs was studied with microdialysis and radioimmunoassay, and 2) brain mechanisms underlying drug dependence and the development of a new drug for drug dependence

were studied in rats (in collaboration with the Japan Science and Technology Agency). In clinical research, studies examined: 1) the effectiveness and adverse effects of new psychotropic drugs, 2) the involvement of the medial prefrontal cortex in motivation-oriented behavior (functional magnetic resonance imaging study in collaboration with the National Institute of Radiological Science,); 3) nerve growth factors involved in neurodegenerative diseases (genetic research in collaboration with the Institute of DNA Medicine,); and 4) the genetics of akathisia induced by antipsychotic agents.

Psychophysiology group

Studies examined: 1) the effects of “*yokukansan*” and quetiapine on the cyclic alternating pattern as an index of sleep structures,; 2) guidelines for the evaluation and management of chronic insomnia in primary care,; 3) the clinical effects of group cognitive behavioral therapy for outpatients with psychophysiological insomnia,; 4) psychiatric care for patients with obstructive sleep apnea syndrome treated with nasal continuous positive air-way pressure, and 5) the characteristics of sleep disturbance in patients with functionally gastrointestinal disorders.

Psychogeriatric group

Firstly, a study of the neuropsychological evaluation of neurodegenerative disorders using brain imaging modalities such as magnetic resonance imaging and single-photon emission computed tomography, which suggested that a reduction in hippocampal volume in Alzheimer’s disease is related to delayed responses on neuropsychological tasks. Secondly, a longitudinal study of the prevalence of psychiatric disorders in patients with breast cancer was performed.

General hospital psychiatry

In a study of interventional therapy based on cognitive-behavioral therapy aimed at preventing recurrences of depression, a computer system and sleep evaluation methods were introduced in addition to a previous evaluation system for more effective presentations and for more precise estimation, respectively. Furthermore, an investigation of new indications for this intervention for atypical depression, bipolar depression, and patients with insomnia was performed. Another study investigated the issues associated with mental care services for patients with cancer, and their families and medical staff.

Clinical electroencephalography group

Studies examined: 1) adverse properties of psychotropic drugs to reduce seizure threshold and provoke seizures, which might result in the several forms of psychosis in patients with epilepsy; and, 2) characteristics of patients with epilepsy demonstrating social withdrawal. Furthermore, we reported on a patient with symptomatic generalized epilepsy in which long-lasting control was achieved by the addition of zonisamide.

Clinical psychology group

Studies examined: 1) psychotherapeutic processes and the treatment techniques of cognitive behavior therapy, Morita therapy, relief care, and psycho-oncology; and 2) the

characteristics of developmental disorders and higher brain dysfunctions using psychological assessments. Furthermore, we taught graduate students and engaged in the training of residents.

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General Summary

We have 9 subspecialty research groups: 1) the Congenital Metabolic Diseases, Endocrinology, Gastroenterology and Medical Genetics group, 2) the Allergy and Immunology group, 3) the Neurology group, 4) the Hematology and Oncology group, 5) the Cardiology group, 6) the Infectious Diseases group, 7) the Neonatology group, 8) the Nephrology group, and 9) the Pediatric Psychiatry group. The ultimate aim of these subspecialty groups is providing practical benefits to patients and their families by means of basic and clinical research. To accomplish this aim, we must have a high motivation for research and cooperate with each other. Regarding our achievements this year, however, we had fewer publications and reviews than usual. Therefore, we must make efforts to increase our academic achievements next year.

Research Activities

Congenital metabolic diseases, endocrinology, gastroenterology and medical genetics

Accomplishments of our group this year are as follows.

1. We used an anti-CD3 antibody to induce immune tolerance to enzymes in enzyme replacement therapy for lysosomal storage diseases (LSDs).
2. We developed a novel gene therapy strategy for LSDs.
3. We found that endoplasmic reticulum stress was a cause of autophagy build-up in LSDs.
4. We surgically prepared rats with left ventricular heart failure and then analyzed the expression of urocortin 1, 2, and 3 and corticotropin-releasing factor receptor type 2 α in their brains.
5. We analyzed the effects of drugs for treating inflammatory bowel diseases.
6. We showed the usefulness of a kit for diagnosing Norovirus infections.
7. We are developing methods for the molecular diagnosis of genetic diseases by means of comparative genomic hybridization arrays and multiplex ligation-dependent probe amplification.

Neurology

The outcomes of acute encephalopathy in children who underwent rehabilitation at our rehabilitation center were presented. The main sequelae were motor disturbance (in 25%), mental deterioration (in 90%), higher cortical dysfunction (in 80%), and epilepsy (in 70%). The patients were divided into 5 groups according to the clinical courses at onset: patients with metabolic disorders, patients with cytokine storms, patients with status convulsivus, patients with severe refractory status epilepticus, and patients with the main symptom of decreased consciousness. Typical patients of each group were described along with the results of magnetic resonance imaging of the brain and of single photon emission cerebral tomography. In patients with metabolic disorders and cytokine storms, the sequelae were generally severe. In patients with status convulsivus, the main sequela was mental deterioration. In patients with severe refractory status epilepticus, the most controversial problem was epilepsy. In patients with the main symptom of decreased consciousness, higher cortical dysfunction was the main problem.

Allergy and Immunology

We have organized and performed the following multicenter clinical studies: 1) Preventive effect of tulobuterol patch for the long-term management of infantile asthma (PET study); 2) Effect of tulobuterol patch on exacerbations in the management of childhood asthma (PAMC study); 3) A comparison of continuous inhalation of salbutamol and continuous inhalation of isoproterenol for severe pediatric bronchial asthma: A multicenter, double-blind, randomized study (CIT study); 4) Optimal stepdown therapy for controlled pediatric asthma responded to salmeterol/fluticasone propionate (OSCAR study); and 5) Preventive effect of pranlukast on nasal membrane swelling in Japanese cedar pollinosis (PRAN study). Recently, we finished the PET study and have prepared a manuscript (on submission).

We developed a questionnaire for measuring quality of life in caregivers of children with atopic dermatitis²⁹. We are now developing a shorter version of the questionnaire.

Cardiology

For basic research, a rodent model of right heart failure was created to investigate gene expression and physiological changes in right ventricular remodeling. To clarify the effect of right heart failure on each organ, we undertook a collaborative study with the adult cardiology group and pediatric endocrinology group. We also studied the growth of the pulmonary artery in a rodent model of pulmonary artery stenosis created with pulmonary artery banding. For clinical research, we are examining: 1) magnesium kinetics in pediatric cardiology, 2) treatment of pediatric arrhythmia with magnesium, 3) the secretion and kinetics of atrial natriuretic peptide and brain natriuretic peptide in pediatric cardiac diseases, 4) cardiac lesions of Fabry disease, 5) hemodynamics after the Fontan operation, and 6) postoperative antithrombotic therapy in patients with congenital heart disease.

Infectious Diseases

We focus on primary immunodeficiency, infectious diseases, and collagen diseases in

children. We have been studying the new diagnosis and treatment based on our clinical experiences. Our research and development are as follows.

- 1) Genetic diagnosis of primary immunodeficiency syndrome
- 2) Retrovirus gene therapy for X-linked chronic granulomatous disease
- 3) Surveillance of respiratory infection
- 4) Rapid identification of causative pathogen using polymerase chain reaction techniques
- 5) Molecular analysis of drug-resistance genes of bacteria
- 6) Efficacy and safety of vaccines
- 7) Disease activities and prognosis of juvenile idiopathic arthritis and systemic lupus erythematosus
- 8) Effect of molecular intervention against of refractory collagen diseases

Hematology and Oncology

We have investigated refractory severe chronic idiopathic thrombocytopenic purpura (ITP) in collaboration with several major centers in Japan. We performed a survey of the uses of rituximab and could confirm its effectiveness in patients with severe chronic ITP in Japan. In basic research to improve the rate of eye preservation, we investigated the molecular mechanism of the antitumor activity of the cationic porphyrin 5, 10, 15, 20-tetra-(*N*-methyl-4-pyridyl)porphyrin (TMPyP4) in Y79 and WERI-Rb1 retinoblastoma cells. We showed that the antitumor activity of TMPyP4 can be attributed to the phosphorylation of H2AX and p53 (Ser46) and the activation of the mitogen-activated protein kinase signaling pathway. Moreover, TMPyP4 significantly enhanced the susceptibility to irradiation. These findings provide insight into the molecular mechanism of the antitumor effects of TMPyP4. The G-quadruplex structure is a potential therapeutic target in refractory retinoblastoma.

Pediatric palliative care, which is the active, total, and comprehensive care for children with has recently been attracting attention. The principles of pediatric palliative care were reviewed. Challenges in the management of pain for children with cancer in Japan were investigated through a nationwide survey of hospitals and other institutions. The advantages and challenges of providing palliative care in our clinic were reviewed.

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General Summary

We have organized special clinics for selected skin diseases, including viral diseases, neurofibromatosis type 1, atopic dermatitis, psoriasis, patch-testing, and skin cancers. Integrating concentrated clinical efforts and related basic research should contribute significantly to clinical practice.

Research Activities

Psoriasis

Various systemic therapies, including oral cyclosporin microemulsion preconcentrate, methotrexate, and etretinate, in addition to 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 (UV) A, narrow-band 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 examined the incidence of metabolic syndromes as comorbidities of psoriasis. In a special psoriasis clinic, we select patient-based treatments to satisfy patients' demands. New biologic agents, including infliximab and adalimumab, are now available and have started to be used to treat intractable psoriasis. Clinical trials with new biologic agents, including anti-interleukin (IL)-17A antibodies, have been performed. We have organized meetings with a society of patients with psoriasis in the Tokyo area twice a year in the auditorium of our university to enhance their knowledge about psoriasis.

Atopic dermatitis

The pathogenesis of atopic dermatitis has been attributed to a complex interaction among 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 not only on the basis of evidence-based medicine but also on the basis of QOL issues. We try to obtain a precise medical history from each patient and to learn how QOL is impaired. To support this type of approach, we have organized skin-care lessons in the skin-care clinic twice a week and have hosted an atopic dermatitis forum, which includes monthly lectures and group meetings. For basic clinical research, the levels of substance P, IL-31, and thymus and activation-regulated cytokine related to pruritus in atopic

dermatitis are being evaluated according to disease severity. Clinical trials of opioid- κ receptor have been performed.

Malignant skin tumors

We have been studying clinical courses and postoperative outcomes of patients with malignant melanoma, extramammary Paget's disease, squamous cell carcinoma, basal cell carcinoma, malignant peripheral nerve sheath tumor, malignant fibrous tumors, and cutaneous T-cell lymphomas according to established therapeutic guidelines. For the accurate clinical diagnosis of pigmented tumors, we always perform dermoscopic examinations.

Sentinel lymph-node biopsy is performed, especially for patients with stage II or III melanoma. We are participating in collaborative clinical research for maintenance therapy using local injections of interferon- β . We also perform palliative care for patients with advanced cancer.

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 malignant peripheral nerve sheath tumor (MPNST) in patients with neurofibromatosis 1 is 10%, although information concerning the epigenetic abnormality is limited. We have used the methylation-specific polymerase chain reaction (PCR) and real-time reverse transcriptase (RT)-PCR to analyze the methylation status of tumor suppressor genes and cancer-testis genes in established MPNST cell lines. The finding of abnormal expression of several cancer-testis genes and the inactivation of tumor suppressor genes indicates that disarranged methylation and de-methylation 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 antibody against HSV glycoproteins I and II for patients with genital herpes to determine the type of HSV. After the diagnosis is confirmed, suppressive therapies (patient-initiated therapy and episodic therapy) with variciclovir are started to improve the impaired QOL. Surveys of QOL in patients with recurrent genital herpes and drug sensitivities derived from HSV from recurrent genital herpes are also being performed.

2. Herpes zoster and postherpetic neuralgia

Initial treatments for herpes zoster and postherpetic neuralgia (PHN) are performed in this clinic. Famciclovir is the oral prodrug of penciclovir, an agent that has demonstrated antiviral activity against HSV-1, HSV-2, and VZV. Famciclovir has efficacy similar to that of acyclovir or valaciclovir in the treatment of herpes zoster and is now

undergoing clinical trials in Japan. We are evaluating the clinical efficacy and safety of this drug. PHN is a major sequela of VZV infection and impairs QOL. To control PHN, we are prescribing selective serotonin reuptake inhibitors and gabapentin, and the efficacy of other new drugs, including pregabalin, is now being investigated with visual analogue scales and a device that objectively measures pain (Pain Vision PS-2100, Nipro Co., Osaka, Japan).

Human papillomavirus (HPV) infection

In addition to ordinary cryotherapies, topical vitamin D3 and salicylic acid have been used to treat viral warts. Contact immunotherapy using squaric acid dibutylester and CO₂ and pulsed dye laser evaporation have also been used to treat severe, intractable viral warts. HPV typing with the PCR has regularly been performed for condylomas 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

This year, 1,426 patients were treated with lasers in the Dermatology Laser Unit. The Q-switched ruby laser is useful for treating nevus Ota because of its selective photothermolysis. Superficial pigmented lesions, such as senile pigment freckles, are usually successfully treated with a single treatment. 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. 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, atopic dermatitis, prurigo nodularis, vitiligo, and cutaneous T-cell lymphomas. Targeted phototherapy equipment, such as the 308-nm excimer lamp, is also used. Other special clinics, including those for skin care lessons, therapeutic make-up, acne care, mental care, and *kampo* medicine, are open for patients on demand.

Self-assessment

Psoriasis: To improve patients' QOL and treatment compliance, we have selected therapies on the basis of their risk/benefit ratios. Phototherapy with narrow-band UVB

and the 308-nm excimer lamp has been introduced. New biologic agents have been also used to treat patients with severe psoriasis.

Neurofibromatosis: Many patients with neurofibromatosis type I are still being referred to our special clinic. We are now performing inheritance consultation for pediatric patients. Surgical removal of different types of neurofibroma is performed for in-patients and outpatients to enhance QOL. Genetic analysis was performed for MPNST. **Herpes Virus Infection:** Suppressive therapy has been used to improve impaired QOL. Surveys of QOL in patients with recurrent genital herpes and drug sensitivities derived from HSV are also being performed. To control PHN, we are prescribing selective serotonin reuptake inhibitors and gabapentin.

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

Contact dermatitis: Causal chemicals, environmental allergens, drugs, and foods in patients with contact dermatitis, drug eruption are regularly examined by patch testing.

Atopic dermatitis: We have been treating patients according to established guidelines and the degree of QOL impairment. The psychosocial background of patients is also considered. To increase patient understanding, we have been organizing an atopic dermatitis forum, which include, monthly lectures and group meetings. Basic research is focused on pruritogens, such as substance P, IL-31, Th2 chemokines, and thymus and activation-regulated cytokine.

Malignant skin tumors: We have been treating many patients with skin cancers, including melanomas, basal/squamous cell carcinoma, and extramammary Paget's disease, with surgical operations combined with sentinel lymph-node biopsies and chemotherapy.

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

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

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

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Department of Radiology

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General Summary

Department of diagnostic imaging

1. Clinical usefulness of diffusion-weighted imaging (DWI) in cases with rheumatoid arthritis (RA) Clinical usefulness of DWI in the evaluation of the activity of inflammatory synovitis in cases with RA was assessed in comparison with fat-suppressed contrast-enhanced MRI (FS-CE-MRI), which was set as the gold standard. Correlation of 100% was provided in all 60 regions of interest of four patients. It is thought that DWI can substitute FS-CE-MRI in the patient in whom administration of contrast media is contraindicated.
2. Evaluation of the ankle joint with MRI and dual-source CT (DS-CT) By combination of MRI and 3-dimensional display using DS-CT, anatomical and pathological relationship between the tendons and osseous components of the ankle are found to be better evaluated.
3. Coronary CTA using dual-source CT-comparison with fractional flow reserve (FFR) using flow wire-Coronary CTA using dual-source CT shows high diagnostic accuracy of anatomical stenosis diagnosed by coronary angiography, even for high heart rate patients without β -blocker which increases the incidence of side effects of contrast media. For functional evaluation of coronary artery stenosis, FFR is measured using the flow wire. Coronary CTA in comparison with FFR, also shows a high correlation.
4. CT scoring system as a predictor of neck metastasis in patients with head and neck cancer Nodal metastasis is the most important prognosticator in patients with head and neck cancers. We establish "CT scoring system" that mainly consists of criteria of size, shape, extracapsular spread and focal defect, and assess its availability in comparison with surgical specimen of neck dissection.

Division of Nuclear Medicine

1. Multicenter trial confirmed the effectiveness of strontium-89 for palliative pain relief treatment of multiple bone metastases.
 The bone-seeking radiopharmaceutical Sr-89 has been used as a palliative treatment for patients with bone pain caused by bone metastases. Sr-89 is a suitable isotope because it is a pure beta emitter. We obtained Sr-89 imaging with bremsstrahlung in patients 1 week after injection. Imaging of Sr-89 had not been notified before our report.
2. Assessment of strontium-89 combined therapy with iodine-131 for thyroid cancer with bone metastases.

Bone metastasis in patients with thyroid cancer is obstinacy disease. We have employed I-131 therapy for thyroid cancer. Suitable protocol should be assessed to administer Sr-89 combined therapy with I-131 targeted to bone metastases.

Department of Interventional Radiology

1. Investigation of the physical properties of microcatheters smaller than 2.2 Fr
The physical properties of microcatheters with tip diameters of 1.8-Fr to 2.2-Fr were reviewed. We measured tip hardness, the smoothness of the interior and exterior surfaces, the flow rate, flexibility of the guide wire, the ability to maintain shape, resistance to kinking, visibility, intensity of pulling, and pressure resistance. The apical flexibility of the catheters was good, but flow rate, visibility, and pressure resistance were problematic.

The division of radiation therapy

1. Clinical evaluation of simultaneous cancer with head and neck cancer
Simultaneous cancer is relatively difficult to treat. It is important to consider the site, degree of malignancy for each cancer, and past history, to determine treatment methods and strategies.
2. Clinical evaluation of shorter fraction radiotherapy after breast conserving therapy(BCT) Whole breast irradiation after BCT is necessary for treatment of breast cancer, but radiation period is long. Shortning fraction radiotherapy need only 3.5 weeks, and expected to effect the same local control and acute reaction comparing with standard schedule radiotherapy. Efficacy of shorter period radiotherapy evaluated.
3. RCT of Tri-modality with I-125 brachytherapy and EBRT and short or Long term Hormonal Therapy for High-risk localized Prostate Cancer Long term hormonal therapy has been regarded as inevitable in the treatment of high risk prostate cancer. So this randomized trial (TRIP study) has planned to show the effects of long term AHT with tri-modality radiotherapy. This study might reduce unnecessary side-effect by adjuvant hormonal therapy in patients with high-risk localized prostate cancer.
4. New strategy, IMRT for head and neck cancer IMRT could spare spinal cord, optic chiasm, and parotid gland without compromising target coverage, and improved the target dose distribution. Especially, for locally advanced head and neck cancer closed to organ at risk, IMRT is useful method due to its ability to spare adjacent normal tissues with acceptable target dose uniformity. This method may be useful in re-irradiation of previously irradiated area.
5. Effectivity of relative signal intensity in the diagnose of prostate cancer
We hypothesize that the relative signal intensity (rSI), defined as the ratio of signal intensity of prostate lesion to the signal intensity of the lesser pelvic space, have clearer image and is similar to pathological prostate. 17 patients with pathologically proven prostate cancer underwent DWI (b value 1,500) using a 1.5 T-system. We made rSI and fused rSI images with T2WI. Each rSI images depict tumor more clearly than conventional DWI. And $rSI > 3SD$ was more correlated to pathological prostate cancer area.

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Department of Surgery

Division of Digestive Surgery

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General Summary

Research is an important mandate for academic surgical divisions, and such activities are the mainstays of academic surgeons. However, the increasing demands of clinical practice and administrative duties may reduce the time available for surgical research. As a result, there is growing concern that research activity in the current healthcare climate is becoming increasingly difficult. To compare our research productivity from April 1, 2009, to March 31, 2010, with that of the University of Tokyo and Keio University, we performed an advanced Internet search (Medline). During this period, The Jikei University, the University of Tokyo, and Keio University produced 178, 506, and 597 English papers, respectively. Adding “surgery” to the search as a key word resulted in 72, 19, and 156 publications, respectively. The contribution of surgery to the research activities of The Jikei University appears relatively high, although search results do not mean that the article in the field of surgery came exclusively from the departments of surgery. To maintain this level of research activity, further efforts are necessary.

Research Activities

Upper gastrointestinal surgery

We evaluate the pathogenesis of primary esophageal motor functional disorders, especially achalasia and gastroesophageal reflux disease, using manometry and multichannel intraluminal impedance pH-metry, and we perform many laparoscopic operations. For esophageal cancer, we continue to assess the viability of the gastric tube using an intraoperative thermal imaging system. The correlation between suitable construction techniques and postoperative complications of esophageal substitutes was then investigated. In new research, we are trying to clarify the mechanism of deglutition disorders after esophagectomy from the viewpoint of esophageal motility. Basic research in esophageal cancer led us to identify molecular markers indicating patients' prognoses. We aim to investigate the significance of small ubiquitin-like modifier 1 expression as a prognostic factor in esophageal cancer.

For early gastric cancer, we studied the validity of the limited operation using sentinel lymph node surgery by infrared endoscopy. Sentinel lymph node surgery for gastric cancer was approved as an advanced medical technique in June 2009 by the Ministry of Health, Labour and Welfare and its indications have been extended to include remnant gastric cancer and duodenal neoplasms. Studies of postoperative chemotherapy for advanced gastric cancer are continuing as a Stomach Cancer Adjuvant Multi-institutional Trial Group trial, a competitive examination about the effectiveness of TS-1+ lentinan, and a study of the effectiveness of intraperitoneal injection of paclitaxel. In addition, for patients who show a good response to TS-1, we are performing pathological research on gene expression to explore effective individualized treatments. Postgastrectomy syndrome impairs quality of life. We have been performing function-preserving gastrectomy, reconstruction with a substitute stomach, and limited gastric resection to minimize postgastrectomy syndrome. We have also developed multiple postoperative gastrointestinal function tests to evaluate various gastrectomy procedures and to obtain helpful information for diagnosing or treating postgastrectomy syndrome.

Colorectal surgery

To improve the quality of laparoscopic operations, we are evaluating the usefulness and reliability of the Virtual Reality Surgical Simulator (VRSS) for laparoscopic colectomy. Comparative studies of stress between open and laparoscopic surgery are ongoing. For chemotherapy, we are participating in national multicenter trials to provide new evidence to the world. Moreover, our original regimen is being developed in collaboration with the Division of Oncology/Hematology, Department of Internal Medicine. For the diagnosis of colorectal cancer, a joint project was started with the Department of Radiology, and the usefulness of diffusion magnetic resonance imaging for detection of lymph node metastasis has been reported.

There has been no breakthrough in basic research on various antibodies in relation to cancer. However, we can report the following. 1) The efficacy of indoleamine 2, 3-dioxygenase (the enzyme that leads to cancer immunotolerance) as a useful factor for predicting the recurrence of early colorectal cancer has been reported. 2) We are using enzyme-linked immunosorbent assays to examine the relationship between the reactions of various immunoglobulins in the serum of patients with cancer and several other factors relevant to cancer stage. 3) In joint research with the Department of Urology, we are developing proteomic methods to identify cancer-associated proteins in colorectal, esophageal, gastric, pancreatic, and liver cancers.

Injections of aluminum potassium sulfate and tannic acid have been used to treat anorectal diseases. Functional analysis of anorectal function with stationary 3-dimensional manometry has been introduced for the first time in Japan, and a systemic treatment strategy for anorectal diseases is being developed.

Hepatobiliary and pancreatic surgery

Our main areas of research are as follows: 1) living donor liver transplantation (LDLT) and regenerative medicine, 2) chemotherapy for pancreatic and biliary cancer, 3) expansion of surgical indications for multiple hepatic tumors, 4) laparoscopic surgery for the

liver, gallbladder, pancreas, and spleen, and 5) navigation surgery for liver surgery. The first LDLT was successfully performed for a patient with postnecrotic cirrhosis and hepatocellular carcinoma on February 9, 2007. Our eighth LDLT was performed for a patient with primary sclerosing cholangitis on October 2, 2009. All 8 recipients were discharged in good condition 15 to 33 days after surgery, and all donors were discharged 9 to 13 days after surgery and have returned to their preoperative status. We are planning to extend the indications of LDLT to include acute hepatic failure and ABO-incompatible cases. Our ongoing research on regenerative medicine, such as artificial bile duct made with cultured human bile duct epithelium, may be extended further for preclinical studies. We have carried out a translational research study of combination chemotherapy with 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. Other clinical and experimental trials are ongoing after being approved by the Ethics Committee of The Jikei University.

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Department of Surgery

Division of Chest Surgery,

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General Summary

The Divisions of Chest Surgery and 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, an evaluation of video-assisted surgery for bullous lung diseases in elderly patients with impaired lung function, an evaluation of video-assisted surgery for thymic tumors, and an evaluation of video-assisted thymectomy for myasthenia gravis.

Our clinical studies are also evaluating new devices and methods, such as narrow-band imaging for the thoroscopic diagnosis of benign and malignant lung diseases, and LaparoSonic 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 and Endocrinology Surgery

With the spread of screening mammography in Japan, the number of cases of ductal carcinoma in-situ has been increasing. We have studied factors involved in the progression of ductal carcinoma in-situ to invasive breast cancer by targeting microinvasive ductal carcinoma.

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

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

The detection of circulating tumor cells (CTCs) 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 CTCs in the bone marrow for survival in patients receiving chemotherapy.

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Department of Surgery

Division of Pediatric Surgery and Vascular Surgery

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General Summary

Pediatric Surgery

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

Vascular Surgery

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

Research Activities

Pediatric Surgery

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.

Clinical studies

1. Endoscopic treatment for vesicoureteral reflux using Deflux
 There are 3 options for managing or treating vesicoureteral reflux. We select endoscopic treatment with a dextranomer/hyaluronic acid gel (Deflux, Q-Med, Uppsala, Sweden). We have treated 3 cases, 2 of which completely resolved.
2. Electrolyte and acid-base balances in laparoscopic surgery
 Carbon oxide alters electrolyte and acid-base balances in laparoscopic surgery.
3. In severe cases of gastroesophageal reflux, a surgical procedure called fundoplication is performed. This procedure is performed laparoscopically in our hospital. With

minimally invasive laparoscopic surgery, pain is minimized, and postoperative recovery is faster. The number of neurologically handicapped children with gastroesophageal reflux has been increasing in our hospital.

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

Basic studies

1. 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.

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

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

3. Plasmapheresis in severe sepsis or septic shock

During sepsis, there is release of various endotoxins from microorganisms which, to a greater or lesser extent, activates cascade systems including release of cytokines, such as tumor necrosis factor alpha and interleukin 6, and complement components. Plasmapheresis is used to remove these factors. We created a rat model of sepsis and evaluated the 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 established and are commercially available, no such stent grafts are available for the treatment of thoracoabdominal aortic aneurysms (TAAAs). Although the surgical death rate following open surgery for the treatment of AAAs is satisfactory, that for TAAAs remains unacceptably high at 15% to 20%, and further improvement is desperately needed. Because TAAA involves one or more visceral arteries, maintenance of visceral perfusion is mandatory while excluding the aneurysm with stent grafts. We have used a custom-made branched stent graft in combination with covered stents (for visceral reconstruction) for the treatment of TAAAs that were deemed to be inoperable because of co-morbid conditions or a hostile thorax/abdomen. Although stent graft repair for TAAAs requires long operative and fluoroscopic times, this treatment is feasible and safe.

2. 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 Stent in the superficial femoral artery

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

Both a global registry and 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 should be reached in August 2009. We are participating in this trial.

4. Clinical study of specific antibody for heparin-PF4 complexes

Heparin is commonly used for anticoagulation in vascular surgery. Heparin-induced thrombocytopenia (HIT) is a rare but life-threatening complication with thrombosis of veins and arteries. Even if heparin use is limited, it occasionally induces the production of specific antibodies against heparin-PF4 complexes. Patients with such antibodies are at increased risk for HIT. The prevalence of these antibodies in patients receiving heparin is presumably underestimated. Accordingly, we prospectively measured antibodies against heparin-PF4 complexes and the activity of PF4 and investigated whether they are related to symptoms of HIT, particularly in patients undergoing major vascular surgery. We measured these variables in 300 patients 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 will be 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 lower extremities becomes longer, and reperfusion syndrome might occur. We have used a small sheath for supplying blood flow to the distal lower extremities and for preventing complete ischemia of the lower extremities and consequent reperfusion syndrome.

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Department of Orthopaedic Surgery

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General Summary

Basic Research

The research performed in our Department of Orthopaedic Surgery, from studies on connective tissue cells to clinical application of basic research data, continues to be held in high regard both in Japan and abroad. This is reflected by the large numbers of papers published in English-language journals and of competitive scientific grants awarded to department members. The number of scientific awards received by our researchers has further increased in the last year, reaching 11 prizes accepted during the last 5 years. The highest priority of our research team is to carry out basic research that is readily applicable to clinical practice, not just that which deals only with basic science itself.

Clinical Research

An approach in which basic studies are carried out with clinical practice in mind and in which clinical research is strongly based on basic science has been proven valid by the results of many collaborative multicenter projects. We continue to cultivate and to present to the world our department's vision that an orthopaedic surgeon dealing with bones, cartilages, blood vessels, ligaments, tendons, and skin shall find answers to the questions of everyday clinical practice through basic laboratory research. This new concept of orthopedic surgical practice has started to be incorporated into evidence-based research: 2 of our researchers have been appointed authors of bone metabolism-related clinical guidelines.

Research Activities

Arthroscopic Bankart repair for recurrent shoulder dislocation in patients older than 40 years

Clinical features and results of arthroscopic surgery were evaluated in patients older than 40 years who had sustained recurrent dislocations of the shoulder without a full-thickness tear of the rotator cuff. Three-fourths of the patients had sustained the first dislocation in their 20s. The mean Japan Shoulder Society Shoulder Instability Score was 51 points before surgery and reached 92 points at the final follow-up. The postoperative limitation of range of motion was decreased with the procedure.

Isolated avulsion fracture of the lesser tuberosity of the humerus in an adolescent tennis player

An avulsion fracture of the lesser tuberosity of the humerus was diagnosed in a 17-year-old tennis player. He began to feel pain on stroking 4 years before admission. Pain was induced by elevation and internal rotation of the shoulder. Arthroscopy showed impingement of a bony fragment between the glenoid rim and the lesser tuberosity of the humerus. The bony fragment was surgically removed, and the subscapularis muscle tendon was repaired.

Spondylolisthesis of the lumbar spine in patients with severe osteoarthritis

We investigated the incidence of spondylolisthesis of the lumbar spine in patients with severe osteoarthritis. Spondylolisthesis was identified in 57% of patients with osteoarthritis of the knee and in 20% of patients with osteoarthritis of the hip. Lumbar disorders should be considered when patients complain of pain around the knee and when severe osteoarthritis is diagnosed with radiographic findings.

Prevention of dislocation after primary total hip arthroplasty with a modular femoral stem

Rotational deformity of the proximal femur remains a common problem after primary total hip arthroplasty in Japanese patients. The deformity can result in postoperative dislocation. The rotation-free modular system used for total hip arthroplasty in Japanese patients produces promising results in regard to preventing postoperative dislocation.

Patient-specific templating method in total knee arthroplasty: A prospective study of the accuracy of different patient-specific bone cutting guides

Preoperative and intraoperative patient-specific templating has gained attention as the next technological development after computer-assisted surgery navigation systems in knee surgery. In our department, we have been evaluating the accuracy of implant positioning during total knee arthroplasty with patient-specific bone cutting guides and are 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 precise preoperative planning software. The comparative analysis on accuracy of different patient-specific cutting guides is being performed in a prospective manner.

Examination of hallux rotation in hallux valgus

We examined rotations of the hallux in patients with hallux valgus by comparing coronal computed tomographic images of these patients with those of a control group of patients and examined the degrees of rotation of the first and second metatarsals and the proximal phalanx of the hallux. Our results indicated that hallux rotation is caused by strain of the soft tissue around the metatarsophalangeal joint.

Collagen cross-linking and abnormalities of bone metabolism in osteoporosis and diabetes

Collagen cross-linking, a major posttranslational modification of collagen, plays many important roles in the biological and biomechanical features of bone. Our recent basic and clinical investigations of collagen cross-links seem to open a new chapter for general medicine as well as for orthopaedic practice. For example, serum or urine levels of pentosidine are now used to estimate future fracture risk in osteoporosis and diabetes. In our research, we demonstrated age-related changes in collagen cross-links in bone and abnormalities of cross-links in osteoporosis and diabetes.

Effects of alendronate on bone formation induced by recombinant human bone morphogenetic protein 2

Local alendronate administration could inhibit β -tricalcium phosphate (TCP) resorption or bone formation or both induced by recombinant human bone morphogenetic protein 2.

Bone formation and resorption was examined in patients after implantation of β -TCP blocks with 60% and 75% porosity in opening wedge high tibial osteotomy. The β -TCP was completely resorbed within 3.5 years.

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Department of Neurosurgery

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General Summary

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

Research Activities

Cerebrovascular 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.

Development of a new endovascular opening system

We performed several clinical and basic research studies regarding endovascular therapy.

Development of a new endovascular operating system

We developed a state-of-the-art endovascular neurosurgery suite that offers integrated neurosurgical and radiological capabilities. A specially designed biplane digital subtraction angiography system was installed in the neurosurgery operating room. In May 2008, 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.

Development of bioactive coils (Matrix coil)

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

Development of Mebiol gel

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

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

Flow dynamics for intracerebral aneurysm

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

Brain tumor

In the treatment of malignant glioma, local recurrence often determines prognosis. The principal of therapy thus becomes the control of local recurrence. However, treating local recurrence with chemotherapy is difficult because the blood-brain barrier is a major obstacle preventing chemotherapeutic drugs from reaching brain tumors. To overcome these problems, a method has been developed for the local sustained release of chemotherapeutic agents by their incorporation into biodegradable polymers. Gliadel Wafer (Eisai Co., Ltd., Tokyo, Japan), which contains carmustine, has been authorized in

Europe and the United States and is used for the patients with malignant glioma. On the other hand, recent advances in liposome technology have shown promise for the introduction of chemotherapeutic agents with reduced toxicity, extended longevity, and potential for cell-specific targeting. In some previous reports, liposomal doxorubicine was used systemically to treat malignant glioma. In our study we have tried to use doxorubicine within thermoreversible polymer for intracranial implantation, a strategy that has been shown to be safe and successful in the treatment of malignant gliomas. We will investigate the release kinetics, toxicity, distribution, and efficacy of this preparation *in vitro* and *in vivo*.

We investigated the safety and clinically effects of immunotherapy with fusions of dendritic and glioma cells with interleukin (IL)-12 in patients with malignant glioma. The subjects were 15 patients with malignant glioma, ranging in age from 40 to 62 years. Dendritic cells were generated from the peripheral blood. Cultured autologous glioma cells were obtained from surgical specimens in each case. Fusions of dendritic cells and glioma cells were prepared with polyethylene glycol. All patients received 3 to 7 immunizations with fusion cells with IL-12 at intervals of 3 weeks. Fusion cells were injected subcutaneously close to a cervical lymph node, and IL-12 was injected transvenously. There were no serious adverse effects, and partial responses have been observed in 2 patients.

Neurotrauma

Few institutions have engaged in research on neurotraumatology. One of the unique aspects of our department is research in this area, which has 3 major topics.

We examined the prevalence of sports-related head injury in collaboration with the Japan Society of Clinical Sports Medicine and the Japan Society of Neurotraumatology.

We also examined sports-related concussion and performed mechanical studies of head-injury through simulations.

Syringomyelia

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

1) Evaluation of the cerebrospinal fluid obstruction at the craniovertebral junction in patients with Chiari malformation In syringomyelia related to Chiari malformation, the relation between cerebrospinal fluid (CSF) circulation blockage and cavitation of the spinal cord has been clarified. Therefore, the improvement of the CSF circulation becomes the goal of surgical treatment. However, the mechanism of cavitation of the spinal cord is not fully understood.

In patients with Chiari malformation, the cerebellar tonsils and the ventral vector (i.e., the dens) compress the spinal cord and restrict CSF circulation. We examined whether these 2 factors influence the effects of foramen magnum decompression.

2) Fluid in the syrinx

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

researching the fluid by measuring cytokine and antibiotic concentrations.

Spine and spinal cord group

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

In clinical research, an analysis of pain in patients with neuropathic pain was started. The DynaCT scanning system (Siemens Medical Systems, 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.

Pediatric Neurosurgery

The Division of Pediatric Neurosurgery, The Jikei University Hospital Women's & Children's Medical Center, was established in October 2002. In the last 6 years more than 1,000 new cases of various entities have been collected and recorded in our data bank, including hydrocephalus (27% of cases), spina bifida (25%), brain tumors (13%), and craniofacial anomalies (8%). Since April 2003, clinical research fellows, 12 from other domestic universities and 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 (*J Neurosurg* 106: 2006). On the basis of these large clinical series with extensive clinical investigations, we have proposed a unique theory for the specificity of CSF dynamics in immature brain, namely "Evolution Theory in CSF Dynamics" (*Childs Nerv Syst* 22: 2006).

We have also completed the development of a new neuroendoscope and proposed a new surgical technique (*J Neurosurg*: 102, 2005) and a specific technique for intracranial cyst (*J Neurosurg*: 103, 2005) 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. Our department has continued as the headquarters of the International Study Group on Neuroen-

doscopy, the Japanese Society for Pediatric Neurosurgery, the Japan Academy of Hydrocephalus Research, and a member of our department has served as the President of the Executive Board Committee of the International Society for Pediatric Neurosurgery and the Japan Association of Medical English Education.

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Department of Plastic and Reconstructive Surgery

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General Summary

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

Research Activities

Gene analysis and staged surgical procedures in patients with syndromic craniosynostosis

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

Gene transfer into limb buds by means of electroporation

Electroporation was used to transfer genes into the extremities of cultured mammalian embryos. Std-ddy mice were anesthetized with ether on embryonic day 11. These embryos, together with the placenta and embryonic membranes, were dissected from the surrounding decidua. The yolk sac, amnion, and chorioallantoic placenta were preserved in Hanks Balanced solution. An injection of 0.1 μ l of pEGFP-N1 vector was performed into the yolk sac. The extremity was grasped with forceps-type electrodes and electroporated with 3 pulses of 30 to 50 V for 50 milliseconds. After the amnion was removed, the embryo was placed in a bottle filled with mouse serum solution. Ninety-five percent O₂ and 5% NO₂ were supplied to the bottle via a tube 4 times a day. The embryo was cultured at 37°C and rotated at 30 revolutions per minute for 24 hours. The placenta was removed, and the embryo was fixed in 4% paraformaldehyde. Frozen sections were prepared with liquid nitrogen and observed with fluorescent microscopy. The specimens demonstrated that green fluorescent protein was present throughout the body after 50-V electroporation but was distributed in a more restricted area after 30-V

or 40-V electroporation. The embryos that were electroporated with 30 V revealed gene transfer localized to the epidermis and dermis.

Distraction osteogenesis

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

Morphologic study of bone conduction mechanisms

Experiments of artificial bone osteoconductivity concern the extremities more often than the cranium. Therefore, we performed an experimental study of osteoconductivity of β -tricalcium phosphate (β -TCP) in a cranial bone defect. Bone regeneration was evaluated in full-thickness circular defects (10 mm in diameter) created bilaterally in the parietal bones of adult female Japanese white rabbits. The animals were divided into 3 groups. In group A, a β -TCP disk (9.5 mm in diameter, 2.0 mm in thickness) was inserted into the bone defect. In group B, granules of β -TCP (approximately 0.1 g) were inserted, and in group C, nothing was inserted. The periosteum was repaired, and care was taken to avoid damaging the dura. Bone regeneration was assessed with macroscopy, roentgenometry, intensity, and histological examination. The results showed that β -TCP has good biocompatibility with cranial bone.

Tissue engineering

Flaps lined with mucosa are in great demand for nasal, oral, tracheal, and urogenital reconstruction. Fascia lined by mucosal tissue have already been developed as a new reconstructive material. Sublingual mucosa was obtained from Japanese white rabbits, and separated mucosal cells were subcultured twice for 4 weeks. The cells were transplanted to the fascia of the femoral muscles in the same rabbits. The fascial tissue was removed together with the muscular tissue 1 week after transplantation. Specimens were stained with hematoxylin and eosin and immunohistochemically stained for cytokeratin, a specific marker of mucosal cells. The growth of mucosal tissue was confirmed with histological examination. Fasciomucosal complex tissue developed. Fascia proved to be a useful scaffold that cross-links the transplanted mucosa and muscle.

Hemodynamic analysis of capillary blood vessels in patients with diabetes

The recent increase in the number of patients with diabetes has led to an increase in the prevalence of diabetic foot gangrene. Amputation either below or above the knee should be avoided as long as possible through the use of both conservative and surgical treatments. However, other than the ankle-arm pressure index and the cardio-ankle vascular index, few effective methods have been reported for predicting diabetic foot

lesions. We found that hemodynamic analysis with video microscopy of blood flow through capillary vessels in the eponychium of the toes in patients with diabetes can indicate the stage of microangiopathy and might be used to predict diabetic foot lesions. The effectiveness of prophylactic treatment using HT_{2A} receptor antagonists will be investigated with this new device.

Functional analysis of desert hedgehog in patients with macrodactyly

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

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Department of Cardiovascular Surgery

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General Summary

The main investigation in our department involved clinical study, evaluation of alterations in cardiac performance and long-term results after corrective surgery, and experimental studies to solve the clinical problems we are facing. Clinical investigations, including follow-up studies, of valvular and ischemic heart diseases were a main area of our clinical research, as were studies of complex congenital anomalies. The recent increase in aortic aneurysms has become another concern in our field. Starting this year we have examined mid-term results, because we had a large enough number of surgical cases in the last few years. New treatment approaches utilizing new surgical techniques, new devices, and research outcomes have been investigated and attempted. We are also performing several experimental studies with in vivo models. The experimental projects include protection of the lung during extracorporeal circulation and postischemic conditioning after cardiac arrest. Our studies were completed and submitted to journals. The major activities are described below.

Research Activities

Basic research

1. Studies of cardiopulmonary protection strategies during open-heart surgery
 - 1) Experimental studies on the prevention of ischemia-reperfusion-induced pulmonary dysfunction after cardiopulmonary bypass with terminal leukocyte-depleted lung reperfusion

Background: We tested the hypothesis that ischemia-reperfusion-induced pulmonary dysfunction after total cardiopulmonary bypass (CPB) is prevented by controlled pulmonary reperfusion with leukocyte-depleted venous (hypoxemic) blood during the early phase of reperfusion.

Methods: Twenty-one, 4- to 5-week-old piglets underwent 180 minutes of total CPB with occlusion of the pulmonary artery (PA) followed by reperfusion. In group I, unconditioned reperfusion was performed by means of simple unclamping of the PA, whereas selective pulmonary reperfusion with either leukocyte-depleted arterial (hyperoxemic) blood (in group II) or venous (hypoxemic) blood (in group III) was applied for 15 minutes at a flow rate of 10 mL/kg/minute before PA unclamping.

Results: Uncontrolled reperfusion in group I caused pulmonary dysfunction, characterized by increased alveolar-arterial oxygen tension difference and decreased static lung compliance and pulmonary vasoconstriction, associated with increases in lung myeloper-

oxidase and interleukin 6 levels and endothelin 1 washout after reperfusion.

Terminal leukocyte-depleted lung reperfusion significantly decreased post-CPB lung dysfunction and vasoconstriction, if hypoxemic blood was used as the perfusate. In contrast, the benefit of terminal leukocyte-depleted lung reperfusion against post-CPB lung dysfunction was nullified by the use of hyperoxemic blood.

Conclusions: Leukocyte-depleted reperfusion with hypoxemic blood protects against ischemia-reperfusion-induced pulmonary dysfunction by limiting endothelial damage, cytokine release, and leukocyte activation.

2) Effect of postconditioning: Experimental study using an in vivo piglet model for the cardiovascular surgical setting on the reversal of myocardial stunning by ischemic postconditioning

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

Methods: Eighteen piglets underwent 90 minutes of ischemia followed by 60 minutes of reperfusion on CPB. In 12 piglets, ischemic postconditioning strategies were applied before aortic unclamping: either 6 cycles of 10-second ischemia/reperfusion or 3 cycles of 30-second ischemia/reperfusion. The other 6 piglets were not treated (control).

Results: Left ventricular systolic and diastolic dysfunction, associated with oxidant-induced biochemical injury, was noted in the control group. In contrast, postconditioning allowed significantly better functional recovery of the left ventricle and less myocardial biochemical injury.

Conclusion: Ischemic postconditioning during the early phase of reperfusion produced prompt myocardial functional recovery with decreased biochemical injury in a piglet model of CPB.

Clinical Studies of Pediatric Heart Surgeries

1. Coagulability and fibrinolytic function in Fontan circulation

We have measured plasma levels of thrombin-antithrombin-3 complex (TAT) as an index of coagulability and α 2-plasmin inhibitor-plasmin complex (PIC) as an index of fibrinolytic function in 20 patients subjected to extracardiac Fontan circulation (mean age at operation, 4.2 years). The levels of both TAT and PIC remained higher than normal for 6 months after surgery, even when warfarin was administered. However, levels then began to gradually decrease and had almost completely normalized by 12 months after surgery. This study suggests that patients might need warfarin as anticoagulation therapy for the first year after Fontan circulation, because of the activated state of coagulability. However, warfarin could be replaced by an antiplatelet agent in patients who show normal results and have no major complications for 12 months after surgery.

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

In 12 patients in whom the staged Fontan procedure was indicated after the bidirectional Glenn procedure, we measured superior vena cava flow, which is equivalent to PA flow in bidirectional Glenn physiology, by means of a transit-flow meter intraoperatively. The measurement of PA flow and pulmonary vascular reserve (PVR), incorporated with

serial volume loading, allowed the assessment of PVR capacity in response to an increase in pulmonary flow to simulate Fontan circulation. The PVR capacity, assessed by the percent reduction of pulmonary resistance in response to increased pulmonary flow, was revealed to be a strong indicator of the post-Fontan outcome and the final central venous pressure (CVP) at Fontan circulation. In 8 patients who underwent the Fontan operation, there was a significant relationship between the actual CVP and the CVP predicted by means of intraoperative simulation.

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

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

Clinical study of adult cardiac surgery

1. Valve disease

1) Reconstruction surgery for complex degenerative mitral valve disease

Limitations and long-term results of the surgical method, based on excess leaflet resection. The basic concept of our approach for the complex degenerative mitral valve disease is to create a smooth clear zone and coaptation surface by removing the excess prolapsed leaflet. In addition to leaflet resection, annular remodeling, which can be achieved with the complete ring, contributes to physiological reconstruction of the mitral valve regardless of the location or area of the diseased prolapsing leaflet. In the case of a wide anterior prolapsed area, leaflet resection with artificial chordae is recommended. Simple and complex prolapse cases did not differ in long-term results.

2) Midterm results with the 19-mm Carpentier-Edwards pericardial bioprosthesis in the aortic position

We have usually used Carpentier-Edwards aortic pericardial valves in patients 65 years or older according to published guidelines. In patients with a small aortic annulus, either annular enlargement or insertion of new-generation prosthetic valves has been proposed to minimize the postoperative residual transprosthetic gradient. Annulus enlargement must be performed in a small percentage of elderly patients with an extremely small annulus if the smallest-sized mechanical or tissue valve can be inserted anatomically. Excellent results have been achieved with bovine pericardial valves in the aortic position both with 19-mm valves and with larger valves. We are comfortable using the 19-mm bovine pericardial valves for elderly Japanese patients.

2. Ischemic heart disease

1) Training of bypass surgeons in off-pump coronary artery bypass graft generation

This is the transitional stage from the conventional on-pump coronary artery bypass grafting (CABG) generation to the off-pump generation. In this situation, many teaching hospitals have their own systems for training resident physicians who are not

fully experienced with conventional CABG. We can train resident physicians with the desk model system and simple operative devices for off-pump CABG.

3. Thoracic aneurysm

1) A safe and simple method of retrograde cerebral perfusion

Although methods of brain protection in aortic aneurysm surgery have already been established, strokes and air embolism after cannulation into the atheromatous aortic arch branches still occur. Such complications could be avoided with retrograde cerebral perfusion at the beginning of circulatory arrest. We have performed cardiopulmonary bypass using ascending aortic and right atrial cannulation with a rectal temperature of 25°C. After the ascending aorta is clamped, cardiac arrest is achieved with an anagradecardioplegic solution. Retrograde cerebral perfusion begins through the cannula for retrograde cardioplegia via the superior vena cava (200–250 ml/minute) to remove debris or air. This simple method is beneficial for brain protection.

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General Summary

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

Research Activities

Gynecologic Oncology

1. Vitamin D receptor polymorphisms and the prognosis of patients with epithelial ovarian cancer

Recently, the vitamin D receptor (VDR) polymorphism FokI was shown to be associated with increased susceptibility to ovarian cancer. We examined whether VDR FokI polymorphisms affect the prognosis of patients with epithelial ovarian cancer. The VDR polymorphisms from FokI in 101 patients with epithelial ovarian cancer were genotyped by sequencing. Overall survival was compared between FokI single nucleotide polymorphisms by means of Kaplan-Meier survival analysis, log-rank tests, and the Cox proportional hazard model adjusted for International Federation of Gynecology and Obstetrics stages, postoperative chemotherapy, histologic type, and the presence of residual tumor. Hazard ratios, adjusted hazard ratios, and 95% confidence intervals were determined. The FokI C/C genotype was associated with a better prognosis than was the C/T or T/T genotype (log-rank test: $P=0.008$; adjusted hazard ratio: 0.16; 95% confidence interval: 0.05 to 0.57; $P=0.004$). Thirty months after surgery, 90% of patients with the FokI C/C genotype were still alive; in contrast, 66% of patients with the C/T or T/T genotype were alive. When the cancer stage was restricted to II to IV, 84% of patients with the FokI C/C genotype were still alive: in contrast, only 50% of patients with the C/T or T/T genotype were alive. These results suggest that the VDR polymorphisms from the FokI genotype are associated with the improved prognosis of patients with epithelial ovarian cancer.

2. Integrated Copy Number and Expression Analysis of Chemoresistant Ovarian Carcinomas

Women with serous ovarian cancer are often intrinsically refractory to platinum-taxol-based treatment or become resistant upon relapse. Because the response to chemother-

apy cannot be accurately predicted, we sought to identify somatic DNA copy number variation (CNV) associated with primary resistance in advanced-stage disease. The genome-wide frequency and the level of CNV in 118 ovarian tumors were measured with single nucleotide polymorphism microarrays. A well-defined subset of 85 advanced-stage serous tumors was then used to relate CNV to primary resistance to treatment. The discovery-based approach was complemented by quantitative polymerase chain reaction analysis of copy number of 12 candidate genes previously reported to be associated with clinical outcome in ovarian cancer. Likely CNV targets and tumor molecular subtypes were further characterized with gene expression profiling. Amplification of 19q12, containing cyclin E (*CCNE1*) and 20q11.22-q13.12, mapping immediately adjacent to the steroid receptor co-activator *NCOA3*, was significantly associated with a poor response to primary treatment. On the basis of previously reported copy number associations with outcome, only the amplification status of *CCNE1* was validated as a marker for primary chemoresistance. Chemoresistant tumors with high *CCNE1* copy number and protein expression were predictably associated with increased cellular proliferation, as were a subset of treatment-responsive patients, suggesting a cell-cycle-independent role for *CCNE1* in modulating chemoresponse. Patients with poor outcomes but without *CCNE1* amplification overexpressed genes involved in extracellular matrix deposition. Our findings have identified 2 distinct mechanisms of primary treatment failure in serous ovarian cancer, involving *CCNE1* amplification and enhanced extracellular matrix deposition.

3. Mesenchymal-to-epithelial transition during the formation of inclusion cysts from human ovarian surface epithelium

Most surface epithelial-stromal tumors of the ovary are thought to arise from epithelial inclusion cysts. Thus, these cysts are the precursor lesions of ovarian carcinoma. On the basis of this hypothesis, we aimed to characterize human ovarian surface epithelium in which mesenchymal-to-epithelial transition occurs in the process of inclusion cyst formation. We examined specimens from 9 patients with endometrial cancer who underwent hysterectomy and bilateral salpingo-oophorectomy. Immunohistochemical studies were performed of 10 healthy ovaries containing 92 inclusion cysts and 4 healthy fallopian tubes to examine the expression of antigen markers, including calretinin, podoplanin, D2-40, thrombomodulin, human bone marrow endothelial 1 (HBME-1), vimentin, epithelial membrane antigen (EMA), Wilms tumor 1 (WT1), CA125, MOC31, TAG-72, Ber-EP4, and E-cadherin. We found that the staining rates for mesothelial markers in healthy ovarian surface epithelium were 100% (10 of 10) for calretinin, 80% (8 of 10) for podoplanin, 80% (8 of 10) for D2-40, 70% (7 of 10) for thrombomodulin, 100% (10 of 10) for HBME-1, and 100% (10/10) for vimentin. Staining rates for epithelial markers in tubal epithelium were 100% (4 of 4) for HBME-1, 100% (4 of 4) for vimentin, 100% (4 of 4) for EMA, 75% (3 of 4) for TAG-72, and 100% (4 of 4) for Ber-EP4. Staining rates for markers of both types in inclusion cysts were 51.1% (47 of 92) for HBME-1, 44.6% (41 of 92) for vimentin, 65.2% (60 of 92) for TAG-72, and 88.0% (81 of 92) for Ber-EP4. Ovarian surface epithelium has characteristics of both mesenchyme and epithelium. In contrast, inclusion cysts gain epithelial characteristics and lose mesenchymal characteristics. These findings support the notion that a

mesenchymal-to-epithelial transition occurs during the formation of inclusion cysts from ovarian surface epithelium.

4. Cytokine gene expression signature in ovarian cancer

Host defenses against tumors are controlled by several immunological mediators, including cytokines, that play important roles in the host-tumor immune system conflict. Alterations of cytokine expression and an imbalance in the T helper (Th) 1/Th2 cytokine response have been shown in ovarian cancer. In this study, we sought to clarify whether the cytokine gene expression profile affects the development and progression of ovarian cancer.

5. A randomized study of retroperitoneal closure

A randomized study of retroperitoneal closure versus opening after lymphadenectomy for both uterine cervical cancer and endometrial cancer has been completed because 200 cases were registered.

A clinical study of robotic surgery using the da Vinci Surgical System has been started.

Fetomaternal Medicine

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

Furthermore, we examined the presence of antiphospholipid antibodies in patients who had these obstetrical complications, concerning no risk patients.

2. The effect of antiphospholipid antibodies in unexplained infertility

Patient who experience recurrent pregnancy loss often become infertile or tend to have repeated spontaneous abortions after treatment for infertility. Those transitional conditions have not been noticed so far. We investigated the differences in possible causes and clinical status among these conditions from the perspective of reproductive failure.

Reproductive endocrinology

CD147 expression in implantation-stage endometrium

Many aspects of how pregnancy is achieved have been clarified with the development of assisted reproductive technologies, but the implantation period remains unclear.

CD147 is expressed at high levels on the surfaces of various tumor cells, stimulates matrix metalloproteinases (MMPs), and plays an important role in successful implantation. A unique mechanism for focal MMP expression may exist in endometrium during the human implantation period, but this remains unknown.

The aim of this study was to determine the expression and hormonal regulation of the CD147 gene during the human implantation period in controlled ovarian hyperstimula-

tion cycles.

Levels of CD147 and MMP2 messenger RNA in human endometrium were significantly decreased in the secretory phase of controlled ovarian hyperstimulation cycles.

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General Summary

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

Research Activities

Urologic oncology

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

- 1) Proteomic analysis of a new biomarker for prostate cancer and urothelial cancer
- 2) Establishment and biological analysis of a new prostate cancer model derived from Japanese patients
- 3) Research on prostate cancer stem cells
- 4) Research on antisense therapy for prostate cancer

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

- 1) Study of seeds and hormones for intermediate-risk prostate cancer
- 2) Clinical study of high dose rate brachytherapy with external beam radiation therapy for high-risk prostate cancer
- 3) Histopathological study of specimens from radical prostatectomy
- 4) Study of deep venous thrombosis after urological surgery
- 5) Study of the incidence of latent prostate cancer
- 6) Evaluation of the early detection with computed tomography of bone metastasis from renal cell carcinoma (RCC)
- 7) Study of small-incision surgery for RCC
- 8) Study of cryosurgery for small RCC

Neuourology and Urodynamics

1. Epidemiological study of dysuria
2. Study of quality of life in benign prostatic hypertrophy

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Department of Ophthalmology

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General Summary

The main research interest of our department is the pathophysiology of the visual processing system. The following topics are the subjects of basic and clinical studies: cataract, neuro-ophthalmology, ocular oncology and histopathology, biochemistry, functional magnetic resonance imaging (MRI), 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 to be performed through incisions of 2.4 to 3.0 mm. Surgeons are now experimenting with even smaller incisions. We began using a standard phacoemulsification and aspiration (PEA) device to perform bimanual PEA 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 could safely implant a hydrophobic acrylic single-piece IOL through a 1.8-mm incision. We are able to choose various premium IOLs, for example multifocal IOLs, toric IOLs, and yellow IOLs. We implant these new IOLs and evaluate visual functions with them.

Neuro-ophthalmology

1. Optic neuropathies caused by toxic substances, especially ethambutol, were explained in detail in the section on optic pathway disorders of *Management Manuals for Serious Adverse Effects* published by the Japan Pharmaceutical Information Center.
2. Neuro-ophthalmological pathophysiology was reviewed in a textbook for medical students on receptors for vision and the visual pathways, light reflex, near response (convergence reflex), corneal reflex, nystagmus, choked disc, optic neuropathy, and perimetry and visual field defects. An outline of general ophthalmology was prepared for a textbook for pharmacology students on visual disturbances and diagnostic approaches to eye diseases.

3. We reported a case of pediatric optic neuritis with thalamic lesions (clinically isolated syndrome), cases of optic neuritis associated with hypophysitis, pupillary responses to blue light stimulus in macular detachment using a novel computerized pupillometry, and clinical features of anti-aquaporin 4 antibody-positive optic neuritis.

Ocular oncology and histopathology

We reported rare cases of subretinal giant hematoma improved by spontaneous hemorrhage into the vitreous cavity, a mixed tumor of the lower eyelid, compressive optic neuropathy due to an intraorbital tumor associated with von Hippel-Lindau disease, and a tumor of the lacrimal drainage system.

Glaucoma

1. The purpose of treatment in glaucoma is to maintain visual function, and clear evidence for the success of treatment is a reduction in intraocular pressure (IOP). We have used eyedrops as medical treatment. Operative treatment is usually performed when glaucoma is resistant to medical treatment. On the other hand, the operation for glaucoma changes the shape of the cornea, exacerbates astigmatism, and decreases the quality of vision. Astigmatism can be divided into types that can or cannot be corrected with lenses (eyeglasses). Therefore, examining which type of astigmatism is increasing is important. A device for analyzing the shape of the cornea was recently developed and has allowed astigmatic quality to be measured in greater detail. We examine corneas with the Orbscan (Bausch & Lomb, Rochester, NY, US) and OPD-Scan (Nidek Co., Ltd., Gamagori, Japan) devices.

2. Because glaucoma requires long-term treatment with eyedrops, patient compliance is important. Beta-adrenergic receptor antagonist eyedrops have been used. Twice-daily treatment has been standard, but several types of once-daily eyedrops that can decrease IOP have recently been marketed. However, eye stimulation and foggy vision became problems, because once-daily eyedrops are gels. Some agents, such as carteolol hydrochloride durability drops (Mikelan LA, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), contain alginic acid, which leads to fewer side effects (stimulation and foggy vision). Therefore, we examined the effects of a change from twice-daily carteolol hydrochloride drops to once-daily carteolol hydrochloride durability eyedrops on IOP and the feelings of use in a patient with glaucoma. The change in eyedrops improved patient compliance and convenience, but no significant change in IOP was noted until 6 months later.

3. Numerous studies have shown that IOP measured with the subject in the sitting position is high in the morning and low in the afternoon and evening. The IOP increases by as much as 2 to 6 mm Hg when the subject, either a healthy person or a patient with glaucoma, lies flat. 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 subject 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 treatments were available that could specifically

decrease the supine IOP, resulting in less fluctuation in IOP due to postural change. However, the agents timolol maleate, latanoprost, and brinzolamide lower IOP in both the sitting and supine positions but do not alter the response of IOP to postural change. The postural response is 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. By means of a pneumatonometer, IOP was measured after the subject had been in the sitting position for 5 minutes and after the subject had been in the supine position for 10 minutes. The sitting IOP and the 10-minutes supine IOP were 10.2 ± 3.3 mm Hg and 13.7 ± 4.5 mm Hg, respectively. The difference between 10-minutes supine IOP and the sitting IOP ($\Delta\text{IOP}_{10\text{min}}$) was 3.43 ± 1.8 mm Hg ($p < 0.05$). There was a significant correlation between sitting IOP and $\Delta\text{IOP}_{10\text{min}}$ ($r = 0.66$, $p < 0.0001$). The lower the sitting IOP was, the lower $\Delta\text{IOP}_{10\text{min}}$ was.

Functional neuroimaging

Patients with glaucoma were examined to confirm several structural changes in the visual pathway by means voxel-based morphometry. The chiasm was evaluated with 3-dimensional T1-weighted images, and the structure of the optic radiation was evaluated with diffusion tensor imaging. Both types of images were acquired with a magnetic resonance scanner. Ten patients with glaucoma and 10 age-matched healthy volunteers were recruited. A significant decrease in signal strength was observed with voxel-based morphometry in areas corresponding to the optic chiasm and the optic radiation in patients with glaucoma. Thus, these results suggest that structural changes occur in the visual pathway of the intracranial structures as well of the eyes in patients with glaucoma.

Developmental functional abnormality

Binocular summation on the visual cortex was explored with postoperative functional magnetic resonance imaging (fMRI) in 2 patients with strabismus. One patient, in whom central stereopsis was preserved, demonstrated binocular summation at the foveal projection area and a peripheral 4-degree projection area. Another patient, who lacked central stereopsis, demonstrated binocular summation not at the foveal projection area. This result suggests that cortical suppression of the foveal projection area was present in the latter patient.

Visual neuropsychology

1. Review articles on visual psychology and neuro-ophthalmology

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

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

We extended the measurement to subjects with retinitis pigmentosa. Our results were the same as in patients with macular degeneration; there was no large-scale remapping

in the adult human V1. Our results support vision-restoring therapies that rely on the stability of the human V1.

3. Publishing 3 papers in international journals

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

Vitreoretinal diseases

We have used a 23-gauge and 25-gauge transconjunctival vitrectomy system for treating macular holes, epiretinal membranes, macular edema, and rhegmatogenous retinal detachment. The 25- and 23-gauge sutureless vitrectomy techniques decrease surgical trauma and improve patient comfort postoperatively. 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 system may effectively reduce operative times for selected patients who do not require the full capability of conventional vitrectomy.

To evaluate the clinical efficacy of a 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 a tomographic scanner (Pentacam, Oculus Optikgeräte GmbH, Wetzlar, Germany)

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

Electrophysiology

We are recording electroretinograms (ERGs) to evaluate retinal function for hereditary retinopathy, retinal dystrophy, and macular disease. The ERG waveforms compound the responses from various retinal cells, such as ganglion, amacrine, bipolar, and photoreceptor cells, which are recorded as a single wave pattern. In addition, we perform the examination with 4 types of recording system, such as the Ganzfeld stimulator, multifocal stimulation, color stimulation, and focal macular stimulation. In Ganzfeld stimulation, we record separate responses from cone and rod cells of the retina according to an international protocol. The multifocal stimulator, which reflects cone function, can record the responses of each separate element in 61 areas of the central 30 degrees of the posterior pole and compare with visual field examination. The color ERG records each response to separate long- and middle-wavelength and short-wavelength cones. We have recently obtained a focal macular stimulator. This stimulator can record the retinal function of the central 5, 10, and 15 degrees and can effectively search for unidentified conditions, such as occult macular dystrophy, causing visual disturbance.

In the future, we will evaluate the waveforms recorded from these ERG stimulators and analyze them further with personal-computer programs. Moreover, as we extract single waveforms from retinal cells of a specific type, we will be able to investigate retinal disorders at the cellular level.

Diabetic retinopathy section

We perform subtenon injections of triamcinolone acetonide for outpatients with diabetic macular edema. After injection, a decrease in macular retinal thickness can be observed with optical coherence tomography, but in some cases macular edema recurs 3 months after injection. For cases in which triamcinolone acetonide injection has no effect, we perform vitrectomy. The transconjunctival microincision vitrectomy is performed with a 23-gauge trocar system. The 23-gauge system is used to make a scleral incision that does not need to be sutured. The advantages of such small incisions include decreased postoperative inflammation and decreased surgical stress.

The vulnerability of retinal ganglion cells in diabetes mellitus has been observed in diabetic animal models and in patients. We are evaluating retina function by recording ERGs in patients with diabetes in whom retinopathy is absent on ophthalmoscopy. We measured photopic negative response (PhNR) among wave patterns obtained in cone ERGs and examined the correlation between the PhNR and the duration of diabetes. We are measuring the thickness of the nerve fiber layer with optical coherence tomography and are investigating the correlation between the thickness of the nerve fiber layer and PhNR amplitude or implicit time or both.

Uveitis

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

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

2. Intravitreal bevacizumab for idiopathic choroidal neovascularization

We assessed the long-term visual and anatomical outcomes and safety of intravitreal injection of bevacizumab for idiopathic choroidal neovascularization. The long-term results suggest an encouraging efficacy and safety of intravitreal bevacizumab for idiopathic choroidal neovascularization.

3. Evaluation of S-cone sensitivity with SITA-SWAP in patients with Vogt-Koyanagi-Harada syndrome

We investigated macular function with Swedish Interactive Thresholding Algorithm-short-wavelength automated perimetry in patients with Vogt-Koyanagi-Harada syndrome. Our results suggest that the measurement of the sensitivities of the short-wavelength-sensitive cones (S-cones) with short-wavelength automated perimetry is a sensitive method for evaluating visual recovery in this syndrome.

Macular degeneration

1. Endothelin (ET)-1 is a potent vasoconstrictor peptide whose levels are increased in diseases associated with vascular dysregulation. The pathogenesis of neovascular age-related macular degeneration (nAMD) is poorly understood but may involve vascular dysregulation and vasoconstriction of the nutrient vessels supplying the choroid. To clarify the possible pathophysiological role of ET-1 in the development of nAMD, we examined plasma ET-1 levels in patients with nAMD. We found increased plasma levels of ET-1 in patients with nAMD. Our data suggest that elevated plasma ET-1 is an important risk factor for nAMD and that an ET receptor antagonist might offer a new therapeutic approach to this disease.

2. Intravitreal pegaptanib for nAMD

We assessed the short-term visual and anatomical outcomes and safety of intravitreal injection of pegaptanib for nAMD. The short-term results suggest an encouraging efficacy and safety of intravitreal pegaptanib for nAMD.

3. Photodynamic therapy alone versus photodynamic therapy combined with intravitreal bevacizumab for nAMD without polypoidal choroidal vasculopathy in Japanese patients

We compared 3-month results of 2 treatments — photodynamic therapy (PDT) with verteporfin alone or combined with intravitreal bevacizumab — for patients with nAMD but excluding patients with polypoidal choroidal vasculopathy (PCV), who were presumed to have AMD. After 3 months, PDT combined with intravitreal bevacizumab for Japanese patients with AMD without PCV appeared to be more effective and required fewer treatments than PDT alone.

4. PDT with verteporfin for PCV with good visual acuity

We evaluated the efficacy of PDT with verteporfin in Japanese patients with PCV and good visual acuity. The results show that PDT is effective for patients with PCV and good visual acuity.

Biochemistry

1. We evaluated the anti-inflammatory effects of intravenously administered methoxy polyethylene glycol- (D, L-lactide) (PLA-PEG) stealth nanoparticles encapsulating betamethasone phosphate (BP) on experimental autoimmune uveoretinitis in Lewis rats. Systemically administered BP-stealth nanoparticles reduced the expression of inflammatory cytokines and vascular endothelial growth factor in the retina and choroid. In conclusion, systemically administered BP-stealth nanoparticles should be useful for treating macular edema and choroidal neovascularization in uveitis.

2. We investigated the properties of the immunocytochemical findings of retinal degeneration in a transgenic rabbit with a Pro347Leu rhodopsin mutation. By 12 weeks of age in the transgenic rabbits, there was already a substantial loss of rods and activation of Müller cells. At the same time, rod bipolar cell processes grew into the inner and outer nuclear layers. Rod bipolar cell processes lengthen during photoreceptor degeneration in rhodopsin Pro347Leu transgenic rabbits. These findings may demonstrate inner retinal remodeling associated with photoreceptor degeneration.

Color vision defects and genetic analysis of retinal diseases

1. We performed genetic testing of a woman with X-linked red-green color vision deficiency and deuteranomalous trichromacy. A 22-year-old woman (proband) and her parents were included in this study. A red-green color vision defect was diagnosed with a Nagel type I anomaloscope. Whether the color vision defects were of a mild or severe form was determined with the Farnsworth Panel D-15 test. Genotypes of L and M visual pigment genes were determined with the polymerase chain reaction. The difference in the peaks of absorbance (I_{\max}) between the first 2 visual pigment genes was calculated. The proband and her father were found to have deuteranomalous trichromacy (mild form). Her mother was an obligate carrier of deuteranomaly because she had normal color vision. Molecular genetic analysis revealed that the proband had 2 distinct M-L hybrid genes. The first 2 expressed pigments from each X-chromosome gene array which differed in I_{\max} by 4 nm and 8 nm, respectively. Our results suggest that it is possible to determine the genotype of a female proband with deuteranomaly when both genetic and color vision testing are performed in other family members.
2. We performed clinical and molecular genetic analysis of various inherited retinal diseases, such as retinitis pigmentosa and macular and cone dystrophies. We identified causative mutations in these diseases. To clarify disease haplotypes, haplotype analysis with mutations was compared between family members and control subjects.
3. We investigated the involvement of various genetic factors in Japanese patients with AMD, which is a common cause of blindness in elderly persons of industrialized countries. Over 500,568 single nucleotide polymorphisms of the entire genome were genotyped with the Affymetrix Human Mapping Arrays (Affymetrix, Santa Clara, CA, USA) and TaqMan assay (Applied Biosystems, Inc., Carlsbad, CA, USA). We are now analyzing candidate single nucleotide polymorphisms in Japanese patients with AMD.

Cornea

The cornea group at The Jikei University chooses the most appropriate corneal surgery by discussing the various options with each patient. We perform Descemet's stripping automated endothelial keratoplasty for corneal endothelium dysfunction.

We have adapted several new treatments for all corneal diseases, for example dry eye, corneal infection, corneal injury, hereditary corneal disease, allergic corneal disease, and keratoconus. Automated lamellar therapeutic keratectomy, in which a microkeratome is used to make a lamellar flap, was performed for several patients with corneal opacity. We found that automated lamellar therapeutic keratectomy enables earlier suture removal and induces less astigmatism than does conventional lamellar keratoplasty.

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

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Department of Otorhinolaryngology

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 Hiromi Kojima, *Associate Professor*
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 Mamoru Yoshikawa, *Assistant Professor*
 Minoru Iida, *Assistant Professor*

Takakuni Kato, *Professor*
 Atsushi Hatano, *Associate Professor*
 Nobuyoshi Otori, *Associate Professor*
 Yasuhiro Tanaka, *Assistant Professor*
 Yoshinori Matsuwaki, *Assistant Professor*

General Summary

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

Research Activities

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, we have compiled a database of cases of cholesteatoma surgery performed at our hospital which is used to analyze the condition of patients, select operative methods, and review postoperative outcomes. In regard to hearing loss, we are studying the physiology of the inner ear in metabolic disorders using experimental animal models and collaborating with Shinshu University in the gene analysis of deaf patients.

Approximately 200 middle ear operations are performed annually at our hospital. The several cases of cochlear implantation performed every year have also yielded favorable results. We perform skull-base surgery, including surgery for cholesteatoma in the petrous portion, 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 on the basis of the degree of development of the mastoid air cells. We determine the timing of the removal of indwelling ventilatory tubes by measuring 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 evaluate saccular function in patients with conditions such as vestibular neuritis, Meniere's disease, and dizziness of unknown cause, to facilitate detailed diagnosis and treatment. Moreover, we are examining the prevalence of abnormal saccules as measured with VEMP testing, in the ictal and nonictal phases of Meniere's

disease, and the incidence of VEMP abnormalities according to disease stage. We also perform furosemide-loading VEMP in patients with suspected delayed endolymphatic hydrops as a putative test for endolymphatic hydrops. In addition, we are continuing research on the localization of the vestibular cortex and the projection from the vestibular system to the cerebral cortex by analyzing cerebral blood flow by means of single photon emission computed tomography in conjunction with the department of neurology.

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

Rhinology

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

Head and neck tumors

We perform radical surgery for common advanced cancers (e.g., total pharyngolaryngectomy combined with reconstruction by free intestinal transplantation for hypopharyngeal cancer and total laryngectomy for laryngeal cancer); however, we perform laryngeal conservation surgery (partial hypopharyngectomy combined with reconstruction by free flap and partial laryngectomy) to preserve function, especially vocal functions, and have achieved favorable outcomes in terms of both laryngeal preservation and survival. For conservative therapy and for postoperative treatment for advanced cancer, we perform radiotherapy or concurrent chemoradiotherapy, with cisplatin and fluorouracil, or both and have achieved favorable results. We use narrow-band imaging endoscopy for diagnosis in routine practice and make good use of this technology for the diagnosis and treatment of early-stage mesopharyngeal and hypopharyngeal superficial cancers.

In research on cancer, we are performing studies to apply findings to future studies or

clinical practice. Such basic studies include extraction of DNA from surgical specimens and evaluation of epidermal growth factor receptor expression, a target for molecularly targeted agents. We are planning clinical studies of human papilloma virus expression, which is thought to be involved in the development of mesopharyngeal cancer and oral cancer, and of treatments for various cancers, including vaccine therapy.

Vocal and swallowing functions

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

We have been performing outpatient day surgery for unilateral recurrent nerve paralysis by intravocal fold injection of atelocollagen for many years; however, we are also performing laryngeal framework surgery for patients who are not considered candidates for intravocal fold injection of atelocollagen.

2. Diagnosis and treatment for spasmodic dysphonia: We have been performing botulinum toxin treatment as a first-line therapy for spasmodic dysphonia with the approval of the ethics committee of the university since December 2004. The prevalence of this disorder is increasing; therefore, evaluating methods of diagnosis and treatment is important, and an important future task is the development of surgical methods for patients who do not respond to botulinum toxin treatment.

3. Evaluation and treatment of dysphagia: We collaborate with other departments, such as the departments of neurology and rehabilitation, and engage in teamwork with co-medical staff, such as nurses. We consider therapeutic strategies for clinical conditions by evaluating patients with video endoscopy and video fluorography and are promoting training for swallowing.

Sleep apnea syndrome

We have attempted to construct a system that can deal with patients from various clinical fields besides otorhinolaryngology, including psychiatry, respiratory medicine, cardiovascular internal medicine, pediatrics, and dentistry, and with visiting medical officers. However, because the number of patients visiting our hospital is increasing, novel approaches are required. Thus, we are planning to provide remote medical care and to perform examinations for sleep disorders using “telesomnology,” which is an applied version of an information technology topic covered by the Japanese Society of Sleep Research, starting this year. The clinical research items covered as research concepts include: 1) nasal breathing and the stability of sleep, 2) sleep disturbance associated with allergic rhinitis (pollen allergy), 3) attention-deficit hyperactivity disorder-like symptoms in children with obstructive sleep apnea syndrome (OSAS), 4) physical development of children with OSAS, 5) maxillofacial growth and sleep-disordered breathing in children with the adenoid facies, 6) a new surgical treatment for adult OSAS, integrating

knowledge from many clinical departments, and 7) the development of telesomnology. Sleep has been found to have significant associations with otorhinolaryngologic diseases, such as allergic rhinitis and gastroesophageal reflux disease.

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Department of Anesthesiology

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 Yasushi Mio, *Associate Professor*
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 Kazuhiro Shoji, *Assistant Professor*

Takehiko Nezu, *Professor*
 Masanori Takinami, *Associate Professor*
 Ichiro Kondo, *Associate Professor*
 Shigehiko Uchino, *Assistant Professor*
 Chieko Fujiwara, *Assistant Professor*
 Yoichi Kase, *Assistant Professor*

General Summary

The 2009 academic year is the fifth year that the Department of Anesthesiology was lead by Professor Shoichi Uezono. The functions of the Department of Anesthesiology are innovative quality of patient care, teaching, and research of perioperative medicine, intensive care medicine, and comprehensive pain management. In 2009 we advanced further and achieved much with support from our faculty, the institutional administration, and the dean of The Jikei University. Below we highlight some of our research achievements in 2009.

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, including protected time and clinical access to research cases. Our investigators have been successful each year in obtaining peer-reviewed research grants, such as Grants-in-Aid for Scientific Research and contract grants. The department continues to build on the strengths of several outstanding programs: vascular anesthesia, pediatric anesthesia, regional anesthesia, neuroanesthesia, intensive care medicine, and comprehensive pain management.

Faculty recruitment is targeted at individuals with demonstrated academic and research productivity as well as excellent clinical management and teaching. In 2009, the department was able to invite Dr. Shuya Kiyama from Keio University as an associate professor. Dr. Kiyama is a nationally and internationally recognized expert in clinical pharmacology and an excellent teacher of total intravenous anesthesia.

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

Highlighted are the ongoing research projects for which the principal investigators are faculty members of the Department of Anesthesiology

Dr. Uezono's research focus has been pulmonary vascular physiology and its clinical application for children with single ventricular physiology. Dr. Omi is interested in the development of simulation tools for learning peripheral nerve blocks. Dr. Taniguchi has been interested in temperature regulation during surgery and its effects on postoper-

ative outcomes in patients undergoing head and neck surgery. Dr. Fujiwara and her colleagues examined the usefulness of the pleth variability index, an index automatically derived from pulse oximeter waveform analysis, to optimize perioperative fluid status. Specialists in intensive care medicine (Drs. Takinami, Uchino, and Kase) have remained active in clinical research including: 1) an outcome study of patients who have undergone tracheostomy in the intensive care unit; 2) an international observational study of patients in the intensive care unit who have sustained acute kidney injury; 3) risk factor analysis of postoperative acute kidney injury in patients with severe intraoperative bleeding; 4) a study of the use of citrate as an anticoagulant during hemodialysis; and 5) a multicenter study of polymyxin b affinity columns in patients with sepsis. Dr. Kitahara and his colleagues in the Pain Clinic have continued their efforts to establish gold standards for the objective assessment of pain. He is also assessing the effect of tramadol on various types of chronic pain. Dr. Kojima has been an active member of the national cancer research council on postmastectomy pain syndrome.

Basic science investigations included studies of gene therapy for experimental pulmonary hypertension (Dr. Uezono), studies of the affects of various anesthetics on the release of substance P at the spinal dorsal horn after tissue injury (Dr. Kondo), the development of a new technologies for measuring endotoxin (Dr. Kase), mechanisms of anesthetic postconditioning in myocardial mitochondria (Dr. Mio), and mechanisms of anesthesia-induced neural excitation (Dr. Yasui). Grants-in-Aid for Scientific Research were successfully renewed during the past year by Drs. Uezono and Yasui. Dr. Mio was awarded a new 3-year Grant-in-Aid.

The appended bibliography of the department shows that a wide range of investigative and scholarly activities were conducted over the past year.

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Department of Rehabilitation Medicine

Masahiro Abo, *Professor*
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Keiji Hashimoto, *Assistant Professor*

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Hideki Sugawara, *Assistant Professor*
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Takeshi Kamikubo, *Assistant Professor*

General Summary

The main research topics of our department are rehabilitation programs that facilitate driving resumption, the predictive validity of a new scale, a new computerized assessment software program, a new therapeutic strategy for aphasia, and the effect of changes in regional cerebral blood flow (rCBF).

Research Activities

Programs that facilitate driving resumption

Being able to drive a car is an important element of a patient's sense of participation in society after a stroke. However, driving a car is a complex task that requires a combination of motor, perceptual, visual, sensory, and cognitive skills. Therefore, after a stroke, some patients have difficulties resuming driving because of various physical and neuropsychological impairments.

To investigate the rate of driving resumption and the severity of physical impairment of patients after a stroke, we performed a questionnaire survey. Data were collected on 525 patients (359 male and 166 female) who had been admitted after a stroke to Tokyo Metropolitan Rehabilitation Hospital from April 2006 through February 2008. Driving habits before and after the stroke were surveyed. Also, the Brunnstrom stage, indicating hemiparetic severity, and functional independence measure (FIM) scores, indicating the activity of daily life, were evaluated from available medical records. Appropriate responses were obtained from 216 patients (41.1%). Among the 118 patients (107 male and 11 female) who had been driving a car before their stroke, 42 (38 male and 4 female) resumed driving afterwards. For patients who resumed driving, the mean Brunnstrom stages were 5.4 ± 1.0 for the upper extremity, 5.5 ± 1.1 for the hand, and 5.5 ± 0.8 for the lower extremity; the FIM motor score was 83.5 ± 8.1 , and the FIM cognitive score was 32.8 ± 3.3 . These values were significantly higher than those of patients who did not resume driving. Furthermore, patients who resumed driving were significantly younger than those who did not. Rehabilitation programs that facilitate driving resumption are necessary for younger patients with mild hemiparesis and disabilities of activities of daily living after a stroke. In addition, multidisciplinary assessments should be considered to determine whether patients can drive safely after a stroke. Comprehensive assessments with physical and neuropsychological findings will be needed for making this decision. A system that supports the resumption of driving and includes evaluations of motor function and cognitive dysfunction might be useful for helping patients resume driving a car after a stroke.

Predictive validity of a new scale

The objective of this study was to examine the predictive validity of a new scale, the Revised Version of the Ability for Basic Movement Scale (ABMS II). The subjects of this prospective study were 71 patients who had had a stroke. In addition to the ABMS II score, other predictor variables were age, limb paresis as indicated with the Brunnstrom stage, and functional ability as indicated with the Barthel Index. Pearson's correlation coefficient analysis showed that the state of functional ability according to the 4-week Barthel Index was positively correlated with the total scores of the ABMS II and Brunnstrom stage at all data collection time-points. The results of linear stepwise regression analysis indicated that the "turn over from supine position" at the start of rehabilitation and "remain sitting" items of ABMS II at 2 weeks after onset of stroke, in addition to the 2-week Barthel Index and the 2-week Brunnstrom stage, were significant predictors (88.9%) of functional ability 4 weeks after onset of stroke. This study provides evidence for the predictive value of the ABMS II with regard to functional ability in patients after stroke.

A new software program for assessing cognitive function

Background: The screening of healthy elderly persons is a worthwhile strategy for detecting cognitive impairment at the earliest possible stage. Simple, reliable tests are needed to evaluate cognitive function. We aimed to measure cognitive function with a new software program, the Higher Brain Functional Balancer (HBFB), in healthy elderly subjects. The objective of this study was to examine the effectiveness of the HBFB.

Methods: Forty-eight healthy elderly subjects participated in this prospective study. In addition to the HBFB quotient, variables examined were age, length of education, and total scores of the Mini-Mental State Examination (MMSE).

Results: Pearson's correlation coefficient analysis showed that the state of cognitive function, according to the total scores of the MMSE, were significantly correlated with scores of "Orientation," the "modified Trail Making Test," "Route 99," and "Just Fit" of the HBFB. The results of linear stepwise regression analysis indicated that "Orientation" and the "modified Trail Making Test" of the HBFB were significant predictors of the total scores of the MMSE.

Conclusions: This study provides evidence for the predictive value of the HBFB with regards to cognitive function in elderly persons.

New therapeutic repetitive transcranial magnetic stimulation strategy for patients with aphasia

Four patients with motor-dominant aphasia after a stroke underwent 10 treatment sessions with low-frequency repetitive transcranial magnetic stimulation (rTMS). Each treatment session consisted of 1200 pulses of stimulation to an area homologous to the most activated site on functional magnetic resonance imaging (fMRI) performed before rTMS. Consequently, rTMS was applied to the right frontal lobes of 2 patients and to the left frontal lobes of 2 patients. Treatment improved language function in all 4 patients. Our therapeutic rTMS strategy seems to be a practical approach for neurorehabilitation for patients with aphasia after a stroke.

Purpose: To examine the safety and efficacy of long-term application of rTMS and speech therapy for patients with aphasia after a stroke.

Subjects and Methods: The subjects were 2 patients with motor-dominant aphasia after a stroke. Before rTMS treatment, fMRI was performed with a word repetition task. The site selected for rTMS application was the area homologous to the most activated area on fMRI. Twenty-minute low-frequency rTMS of 1 Hz was applied once per week in the outpatient clinic for 6 months after a 6-day in-hospital application, combined with speech therapy. Language function was evaluated at the beginning and end of the inpatient and outpatient treatment courses.

Results: rTMS was applied to the right inferior frontal gyrus, because the left inferior frontal gyrus was most activated area in both patients. Both patients successfully completed the therapeutic rTMS protocol without any adverse effects. The inpatient and outpatient treatments improved language functions, such as naming and writing, in both patients.

Conclusion: Our protocol of weekly long-term rTMS treatment is a practical therapeutic approach for patients.

Effect of rCBF changes

Objective: The objective of this study was to clarify the effect of changes in rCBF in language-relevant areas of the dominant hemisphere on the rCBF in each region in the nondominant hemisphere in patients with aphasia after stroke.

Methods: The subjects were 27 patients with aphasia who had had their first symptomatic stroke in the left hemisphere. In each subject, we measured rCBF by means of ^{99m}Tc-ethylcysteinate dimer single-photon emission computed tomography (SPECT). The SPECT images were analyzed with the statistical imaging analysis program “easy Z-score Imaging System” and with voxel-based stereotactic extraction estimation. Segmented into Brodmann area (BA) levels, regions of interest (ROIs) were set in language-relevant areas bilaterally, and changes in the relative rCBF as mean negative and positive Z-values were computed automatically. To assess the relationship between the rCBF changes of each ROI in the left and right hemispheres, the Spearman ranked correlation analysis and stepwise multiple regression analysis were applied.

Results: Globally, a negative and asymmetric effect of rCBF changes in the language-relevant areas of the dominant hemisphere was found on the right hemisphere. The rCBF decrease in the left BA22 significantly affected the rCBF increase in the right BA39, BA40, BA44, and BA45.

Conclusions: The results suggest that the chronic increases in rCBF in the right language-relevant areas are due, at least in part, to reduction in the transcallosal inhibitory activity of the language-dominant left hemisphere caused by the stroke lesion itself and that these relationships are not always symmetric.

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Department of Emergency Medicine

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 Kei Ohtani, *Assistant Professor*
 Kenji Okuno, *Assistant Professor*

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

General Summary

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

Research Activities

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

Publications

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Department of Endoscopy

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 Hiroo Imazu, *Assistant Professor*

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 Keiichi Ikeda, *Assistant Professor*

General Summary

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

Research Activities

Pharyngeal, esophageal and gastric malignancies

1. Endoscopic diagnosis in esophagogastric neoplasia

The early detection and accurate diagnosis of premalignant and malignant lesions in the pharynx, esophagus, and stomach are essential for selecting the most appropriate treatments 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. Most recently, we have introduced transnasal ultrathin endoscopy, which is expected to improve patient compliance. This is particularly useful for screening patients from the nonreferral 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. Our goal of our current research is to develop algorithms for NBI technology which allow the histological type and the extent of tumors in gastric carcinoma to be predicted without biopsy. We also aim to evaluate this technology for the early detection of precancerous changes in the specialized columnar epithelium of Barrett's esophagus. Preliminary results have been reported at several conferences and published. On the basis of our findings with magnified NBI, we have also developed a novel classification system for gastric cancer and demonstrated its advantages compared with the conventional diagnostic system in a prospective study.

2) AFI

The AFI endoscopic system has recently been developed to visualize the autofluorescence emitted from the gastrointestinal wall. Theoretically, AFI may allow the detection

of premalignancies or early stage malignancies that do not have a distinct endoscopic appearance. 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.

3) Ultrathin endoscopy (transnasal endoscopy)

Ultrathin endoscopes can reduce discomfort during endoscopic examination. However, ultrathin endoscopes have a lower image resolution than do conventional endoscopes and, therefore, are associated with a higher risk of false-negative results. Accordingly, we found that gastric lesions are detected at a lower rate with ultrathin endoscopes than with conventional high-resolution endoscopes.

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 will be described later.

4) 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 on endoscopic examination. These lesions include those within the gastrointestinal walls, such as submucosal tumors of the esophagus and stomach, and mediastinal and lymph-node lesions. In EUS-FNA, real-time ultrasonographic images are used to precisely guide the biopsy needle into lesions. The tissues obtained with EUS-FNA are immediately examined by a cytologist or pathologist to detect the presence of any malignant cells. We are now evaluating the technical safety and usefulness of this technique.

2. Endoscopic treatment of esophageal and gastric malignancies

With recent advances in endoscopic diagnostic techniques and instrumentation, the indications for endoscopic therapy have increased in the treatment of early gastric and esophageal carcinomas. Research on the following endoscopic therapeutic modalities is now under way to standardize the use of these techniques in the treatment of 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 the use of endoscopic submucosal dissection (ESD) in the treatment of early gastric cancer, based on the histopathological findings of the cancer. We are also evaluating the possible use of EMR in the treatment of gastric cancers, including small, poorly differentiated adenocarcinomas lacking ulceration, well-differentiated adenocarcinomas 30 mm or less in diameter or confined to the mucosa, and carcinomas lacking submucosal microinvasion. Current indications for the use of 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 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 by means of ESD is considered necessary to further develop the use of endoscopic treatment. Development of a series of endoscopic knives and long-lasting submucosal fluid has 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 following endoscopic treatment, by monitoring intragastric pH after endoscopy. A study to evaluate the risk of sepsis and endotoxemia following ESD, using blood culture, is underway.

2) Therapeutic interventions employing innovative endoscopy systems

The multibending scope (M-scope) is a new type of endoscope that provides greater access to poorly accessible sites. 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 regions not accessible with a conventional endoscope. 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 has a special mechanism allowing the forceps to move laterally and vertically, in addition to the multibending function, are being performed to raise the potential of endoscopic therapy. We have also performed several studies using natural orifice transluminal endoscopic surgery, including full-thickness resection, because current endoscopic treatments are directed only at mucosal diseases.

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

Many studies have demonstrated an association between *Helicobacter pylori* infection and the development of gastric cancer. However, many unknown factors still affect this association. Because this department routinely performs endoscopic treatment for gastric cancer, clarifying these factors is important. Experiments on this topic, particularly on DNA methylation due to *H. pylori* infection, have been performed in collaboration with the Department of Gastroenterology, Toshiba General Hospital. We also have been exploring the roles of inducible nitric oxide synthase (iNOS) in the pathogenesis of *H. pylori*-associated diseases and have demonstrated that the eradication of *H. pylori* plays an important role in 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 have been published in Japan and internationally. In addition, we have reported that diverse topographical patterns of *H. pylori*-induced iNOS expression and iNOS gene 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 has improved prognoses. However, this technique is difficult to perform in such locations as the oropharynx and hypopharynx. Metachronous or synchronous cancer in the oropharynx or hypopharynx has become the main factor adversely affecting the prognosis or quality of life of patients with esophageal cancer. Because of the importance of detecting cancer at an early stage, we have found that using magnifying endoscopy in combination with the NBI system has allowed hard-to-find cancers to be detected at an early stage without Lugol chromoendoscopy. A multicenter randomized controlled study of the clinical value of this new combination endoscopy has been performed.

Functional disorders of the upper gastrointestinal tract

The causes of gastroesophageal reflux diseases, including nonerosive reflux disease and gastrointestinal motility disorders, are difficult to identify. It is important to establish methods to evaluate hypersensitivity and dysmotility of the gastrointestinal tract to understand disease pathophysiology and choose effective treatments. Hence, we have developed a new method for 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 using color Doppler endoscopic ultrasonography to study the hemodynamics of the portal venous system in patients with esophagogastric varices. These studies have clarified some of the factors associated with an increased likelihood of esophagogastric varices to recur after endoscopic treatment. When all such factors are identified, we expect to be able to predict and prevent early recurrence 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 in the detection of gastorenal shunts, which can complicate the treatment of esophagogastric varices, and can delineate the shunt in detail. Therefore, this diagnostic system could be extremely useful for selecting patients with esophagogastric varices who are suitable candidates for treatment by interventional radiology and for predicting its efficacy.

Enteroscopy and Colonoscopy

1. Diagnostic techniques

Capsule endoscopy is a breakthrough modality that can be used to detect lesions located in parts of the small intestine unreachable with an ordinary endoscope system. Internationally, capsule endoscopy has been performed for more than 1 million patients before May 2009. It is recommended as a first-line examination to detect diseases of the small intestine. However, because capsule endoscopy is purely diagnostic, we have introduced single-balloon enteroscopy, which allows biopsies to be performed and hemostatic techniques to be applied to hemorrhaging lesions of the small intestine.

The incidence of colon cancer has markedly increased in Japan. In Europe and the United States, studies describing the use of capsule endoscopy for examining the large intestine have been published. The Jikei University is 1 of 6 Japanese hospitals collaborating on studies of capsule endoscopy for screening patients for diseases of the large intestine.

Accurate preoperative evaluation of the degree of tumor invasion is essential for appropriate decision-making and for determining the optimal therapeutic strategy for patients with colonic lesions. Hence, to maximize diagnostic accuracy, we use a magnifying endoscope with NBI or AFI or both along with conventional white-light examination.

2. Research in endoscopic interventions

Surgical resection has been the first choice for treating large, sessile tumors of the colon.

Recently, some colonic lesions have been treated with endoscopic en bloc resection performed with ESD (a standard treatment for gastric 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 current efforts are focused on establishing safe and reliable endoscopic methods for removing large colonic lesions and to start preliminary use of ESD. Additionally, an infrared endoscopy system is used to evaluate the risk of bleeding from vessels on the base of the ulcer produced with ESD.

3. Capsule endoscopy and enteroscopy

Capsule endoscopy is a minimally invasive endoscopic modality that can be used to detect lesions of the small intestine that traditional push enteroscopy cannot reach. In Western countries, capsule endoscopy has recently been recommended as the first-line endoscopic examination for evaluating and managing obscure gastrointestinal bleeding. We have performed capsule endoscopies for 109 patients since Japanese health insurance began covering the modality in April 2007. Our study found that capsule endoscopy should be performed as soon as possible after a patient visits a hospital with a complaint of melena. We are aiming to further improve the diagnostic accuracy of capsule endoscopy in evaluating obscure gastrointestinal bleeding, by re-evaluating the traditional bowel preparation regimen.

Pancreatobiliary endoscopy

1. Diagnosis of biliary and pancreatic diseases

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

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

For diagnosing ampullary tumors of the duodenum, we have characterized in detail the mucosal surface structures in this region using NBI to magnify microstructures and to help determine whether lesions are benign or malignant. Furthermore, convex array EUS is performed to evaluate the depth of tumor invasion. On the basis of these findings, the need for endoscopic papillectomy is determined. Favorable clinical outcomes have been obtained to date.

2. Treatment with endoscopic techniques in pancreatobiliary diseases

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

now evaluating the practicality of this approach.

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

Palliative care

More and more interest is being shown for palliative care. Various techniques have been developed to provide the best quality of life for critically or terminally ill patients. Endoscopic procedures may play an important role in palliative care, especially in supporting food intake. In our department, percutaneous endoscopic gastrostomy is performed when patients cannot maintain sufficient oral intake. Although percutaneous endoscopic enterostomy is usually 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 its usefulness. Kits for placing percutaneous endoscopic gastrostomy developed by us have reduced the frequency of complications associated with percutaneous endoscopic enterostomy placement. To alleviate stenosis due to tumors of the digestive tract or bile duct, we have performed endoscopic ballooning/bougienage and subsequent metallic stenting and have obtained good 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 help improve the quality of life of patients who are not candidates for radical surgery. The cost-effectiveness of these interventions is another benefit.

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Department of Infection Control

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Research Activities

Epidemiological research on sexually-transmitted infections

From 2003 through 2008, a study group of the Ministry of Health, Labor and Welfare on sexually-transmitted infections was active with Dr. Onodera as the chief investigator. The main objectives of this study group were to perform research to develop methods of preventing the onset and spread of sexually transmitted infections and to promote countermeasures against them. The main study items were: 1) epidemiological research on trends in the onset of sexually transmitted infections, 2) early detection of sexually transmitted infections in young people and trials related to treatment, 3) development of rapid and highly precise testing methods for genital herpes and genital warts, and 4) surveillance of drug-resistant gonococcus and the development of diagnostic and therapeutic methods for gonococcal infections of the throat. Starting in 2009, a new study group, "Study group on development of a system for prevention and treatment of sexually-transmitted infections" was established on the basis of a 3-year plan. The new group undertook further investigations of the same items as in the previous study group. New research starting this year included studies of the establishment of syphilis notification criteria, a behavioral background survey on the diversity of sexual behavior, and a microbiological analysis of the pathogens of sexually-transmitted infections.

According to a survey of trends in the onset of sexually transmitted infections, decreases in the rates of sexually-transmitted chlamydia infections and gonococcal infections have been observed in both men and women in Japan since 2003, but the prevalence of genital herpes and genital warts remains about the same or only slightly decreased in both men and women. A survey of all sexually transmitted infections was performed in model prefectures as an epidemiological survey to verify the fixed-point surveys. Chiba, Ishikawa, Gifu, and Hyogo prefectures were asked to cooperate in the survey for 4 years starting in 2006, and Iwate, Ibaragi, and Tokushima prefectures were asked to cooperate for 3 years starting in 2007. As a result, the analysis was performed only for medical institutions surveyed for 3 consecutive years, but no major differences from all-case reports and trends in the past were found. In all 7 prefectures, monotone decreases were seen in the onset trends survey of gonococcal infections and genital herpes, but in all-case surveillance continued for 3 years, clear decreases were seen, except for male genital herpes.

Self-testing kits for chlamydia infections (polymerase chain reaction method) continued to be distributed by post to young people, but the positivity rates for sexual chlamydia infections did not exceed 3% this year. Responses to a sexual behavior questionnaire clearly showed the necessity of improvements in medical institutions to eliminate anxiety and doubts among persons with positive test results.

These results suggest it is necessary to establish specific definite standards and surveillance design for fixed-point surveys in the future. The government must maintain good relations with non-governmental organizations and medical institutions to maintain the test and medical examinations to diagnose sexually-transmitted infection in young people.

Pandemic influenza in Jikei University Hospital and Social Welfare Facilities

A study was performed of 954 patients with pandemic influenza diagnosed and treated in The Jikei University Hospital from May through December 2009. Anti-influenza therapy was started within 2 days after onset in 94% of patients. All cases were alleviated, and no deaths occurred. Many severely ill patients were those younger than 9 years or older than 60 years, those with underlying diseases, and those in whom the interval between onset and diagnosis was more than 6 days. These results in our University Hospital suggested that early diagnosis and treatment of influenza are essential.

In 3 social welfare facilities where we provided guidance on infection control, we investigated whether our countermeasures for infection control suppressed the spread of influenza. Employees were prohibited to work for 3 days when their family members contracted pandemic influenza. When influenza broke out in the facilities, we discussed with physicians whether persons contacted closely with the patients needed to take preventive medications. Preventive medicine against pandemic influenza was administered after consent was obtained from family members. We could prevent influenza brought into the facilities. And the spread of the infection was suppressed when persons who have contracted pandemic influenza in the facilities was left the facilities.

Clinical characteristics and treatment of urosepsis

Forty-five patients with urosepsis were examined at the Kanagawa Prefecture Shiomidai Hospital. More than 80% of the patients were elderly or had underlying diseases. The efficacy rate of early treatment was 48.9%, and the causes of treatment failure included inappropriate doses and an insufficient number of treatments with antibacterial agents. Three patients died, and early treatment was ineffective in all of them. It is important to administer adequate doses of antibacterial agents in an appropriate dosage regimen in the early treatment of urosepsis.

Toxoplasma encephalitis in patients without human immunodeficiency virus (HIV) infection

The incidence of toxoplasma encephalitis, a characteristic disease of acquired immunodeficiency syndrome (AIDS), has recently increased in patients with AIDS. However, toxoplasma encephalitis also occurs in patients without AIDS, including those who have malignant tumors, have received transplants, and have received immunosuppressants. We investigated toxoplasma encephalitis in patients without AIDS (patients we treated and patients reported on in the literature). Although toxoplasma encephalitis is rare, it must be considered, even in the absence of HIV infection, when multiple intracranial lesions and associated focal symptoms are present in patients who have

underlying diseases, because toxoplasma encephalitis can occur in severely immunodeficient patients.

Basic study of clinical isolates of Pseudomonas aeruginosa (P. aeruginosa)

Minimum inhibitory concentrations (MICs) of 5 carbapenems were measured in 384 strains of *P. aeruginosa* isolated and identified from 282 patients. Among the 384 strains, 11 were multiple drug-resistant strains, and 8 of them produced metallo-beta lactamase. The results of measurement of MIC showed potent antibacterial activity in the order of doripenem, meropenem, biapenem, imipenem, and panipenem. The MIC required to inhibit the growth of 50% of organisms differed by 2- to 16-fold, and the MIC required to inhibit the growth of 90% of organisms differed by 2- to 4-fold. Strains of imipenem-resistant *P. aeruginosa* ($\geq 16 \mu\text{g/ml}$) were isolated at high rates from drains, tubes, catheters, bile, feces, sputum, and catheter urine. *P. aeruginosa* was isolated with high rates in Departments of Rheumatology, Cardiac Surgery, Oncology and Hematology, Neurosurgery, Gastroenterology, Cardiology, and Vascular Surgery.

Biofilms of clinically isolated staphylococci

Staphylococci form biofilms on the surfaces of medical devices, such as vascular indwelling catheters and artificial joints, and lead to chronic infections. Analysis of biofilms formed by clinical isolates of staphylococci is necessary to establish methods for preventing and treating biofilm infections. Since staphylococci in liquid culture media could form biofilms on polystyrene surfaces *in vitro*, we examined the rate of biofilm formation in staphylococcus isolated in Jikei University Hospital. Biofilms were formed in 29.2% of methicillin-sensitive *Staphylococcus aureus* strains (7 of 24 strains), 29.2% of methicillin-resistant *S. aureus* strains (7 of 24 strains), and 25.0% of *S. epidermidis* strains (7 of 28 strains). Biofilms formed by each staphylococcus had different degradation profile by polysaccharide-degrading enzymes, proteolytic enzymes, and/or DNase. These results suggest that biofilms are formed by polysaccharides, proteins, or extracellular DNA. Specially, since biofilms formed by 4 of the 7 clinically isolated strains of *Staphylococcus epidermidis* could be degraded by polysaccharide-degrading enzymes, polysaccharides should be included in the biofilms.

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General Summary

Clinical studies of temporomandibular disorders

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

Clinical and basic studies of obstructive sleep apnea hypopnea syndrome

Our clinical study using X-ray cephalometry analysis indicated a correlation between the e-s (ES) angle and the apnea-hypopnea index (AHI). Our goal is to establish a screening evaluation using the measurement of facial morphology. In an animal study, fat was deposited, particularly in the tongue muscles, by a high-fat diet.

Basic studies of oral mucosal keratinocytes

We examined the expression of T-cell-specific adapter protein (TSAd) messenger RNA and protein in both human and murine oral mucosal epithelium and human primary oral keratinocyte cell cultures. The function of TSAd in keratinocytes was also studied by proliferation and migration assays with TSAd^{-/-} mice keratinocytes.

Research Activities

Clinical studies of TMDs

1. Questionnaire survey about the relation between patients with TMDs and working contents in general dental clinics in Tokyo

Purpose: To study relationships between working contents and patients with TMDs.

Methods: A questionnaire that included 4 TMD screening items (J Jpn Soc TMJ 19(2), 2007) and 8 working contents items (time of personal computer [PC] use, commuting, sleeping, meeting, driving, handwork, heavy-duty lifting, and relaxation before going to bed) was administered to 253 patients who had consulted general dental clinics in Tokyo because of dental problems. Subjects included employed persons, unemployed persons, full-time homemakers, self-employed persons, and students, but this information was not recorded. The Mann-Whitney U-test and logistic regression analysis were performed. As a dependent variable, the results of the TMD screening questionnaire (TMD and non-TMD) were analyzed.

Results: A total 244 patients (120 male and 124 female) completed the questionnaire and were eligible for the analysis. There was no significance difference in mean age between the sexes. The TMD screening questionnaire showed that 35 patients (14.3%) had TMD and 209 patients did not. There was no significant difference in the operation contents

between TMD patients and non-TMD patients. When we assumed that unemployed persons had a commuting time of 0 and compared the operation contents of employed and unemployed persons, we found that employed persons had significantly less PC time, relaxation time before going to bed, and meeting time and that unemployed persons were significantly older. Logistic regression analysis showed a significant odds ratio for PC time (1.84) in women.

Conclusions: In women, the risk of TMD increases with PC time.

2. An evaluation of improvement of symptoms after treatment in elderly patients with TMDs: Comparison between patients 65 years or older and those 39 years or younger To assess the efficacy of our treatment of elderly patients, we performed a retrospective clinical study of 77 patients with TMDs who were 65 years or older and 82 patients 39 years or younger. The results showed that in the older patients, the treatment was effective in 53 joints (68.8%) and that in the patients in whom TMDs were cured, the signs and symptoms had improved within 1 month in 60.4%. In the younger patients, treatment was effective in 59 joints (72.2%), and in patients in whom TMDs were cured, the signs and symptoms had improved within 1 month in 67.8%. According to the classification of the Japanese Society for Temporomandibular Joint, in the older patients the improvement rate of patients with type I disease was highest (92.9%), followed by that of patients with type III-a disease (80.0%) and patients with type II disease (72.2%). The lowest improvement rate was in patients with type III-b disease (45.0%). In the younger patients, the improvement rate of patients with type I disease was highest (100.0%), followed by that of patients with type II disease (77.1%) and patients with type III-a disease (76.9%). The lowest improvement rate was for patients with type III-b disease (43.6%). There was no significant difference between the older and younger patients in the improvement rate of any of the TMD types. The results suggest that conservative treatment, mainly guidance on diet and daily life, is as effective for elderly patients as for younger patients.

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

The committee drafting guidelines for the primary care of TMDs of the Japanese Society for Temporomandibular Joint performed a questionnaire survey of participants of its 20th annual meeting held on July 14 and 15, 2007. Its purpose was to consider strategies for collecting clinical questions from dental care providers. The questionnaire included the position in the society and the social stage, how long the respondent had treated TMDs, the format for clinical questions, and free opinions about the guidelines. An advertising effort was thought to be necessary for the next investigation because we were able to collect only 61 answers. Of the respondents, 54 (89%) were society members and 24 (39%) were authorized specialists. Thirty-one dentists (51%) had treated TMDs for more than 11 years. In the symptoms described in the clinical questions format, pain was the most frequent and was followed by joint noise and limited mouth-opening. The most frequently described treatment was splint therapy, which was followed by mouth-opening exercises and pharmacotherapy. Several answers given by respondents suggested they did not recognize the format style for clinical questions. These results

suggest that we must consider more detailed methods for collecting clinical questions.

Clinical and basic studies of obstructive sleep apnea hypopnea syndrome

1. Cross-validity assessment of the ES angle for the severity of obstructive sleep apnea hypopnea syndrome

Higurashi introduced the ES angle as a method to estimate the severity of obstructive sleep apnea hypopnea syndrome (OSAHS) and reported that its sensitivity and specificity for OSAHS with an AHI of 20 or more are 0.66 and 0.80, respectively. We examined the cross-validity of the ES angle to see whether similar results are obtained even if the subject is changed. The study involved 26 healthy subjects ($AHI < 5$) described by Higurashi and 51 patients with OSAHS ($AHI < 5$) who consulted our department from 2003 through 2009. The subjects underwent lateral cephalometric radiography and overnight polygraphy. We followed the method of Higurashi and measured the ES angle with cephalometric radiography. We examined the validity of the ES angle for the severity screening of sleep apnea syndrome. The median value showed a significant difference between the control group (121.5° ; quartile deviation, $113.3\text{--}125.0$) and the OSAHS group (129.0° ; quartile deviation, $119.0\text{--}135.0$; $p < 0.001$). The criterion-related validity of the ES angle was 0.522. The sensitivity and specificity of an ES angle $> 127^\circ$ for an AHI greater than 20 were 0.63 and 0.69, respectively. The ES angle significantly correlated with AHI, and the cross-validity of the ES angle and AHI was confirmed.

2. Study of adipogenic changes of the lingual muscles in obese rats

Objectives: To study the effects of obesity on the function and morphology of the lingual and masseter muscles related to obstructive sleep apnea, adipogenic changes, and the fiber type composition of the lingual and masseter muscles were examined in rats fed a fatty diet.

Methods: Twelve 8-week-old male Wistar rats were divided into 2 groups. For 10 weeks the obesity group was fed a fatty diet, and the normal group was fed a regular diet. The lingual (genioglossus and geniohyoid) and masseter muscles were removed. The adipogenic change of each muscle was analyzed with Oil O red staining. Expression levels of myosin heavy chain (MHC IIb, IId, IIa, I) messenger (m) RNAs were evaluated quantitatively with real-time reverse transcriptase-polymerase chain reaction.

Results: The body weight was significantly higher in the obesity group (492.7 ± 35.0 g) than in the normal group (326.5 ± 35.1 g, $P < 0.05$). The area of oil droplets was significantly greater in the genioglossus and geniohyoid muscles of the obesity group ($8.2\% \pm 3.7\%$, $7.4\% \pm 2.2\%$) than in those of the normal group ($2.3\% \pm 1.4\%$, $3.0\% \pm 1.2\%$, $p < 0.05$). The oil droplet accumulation was observed in the myofibers of the genioglossus and geniohyoid muscles but not in those of the masseter muscle. The distribution of MHC I mRNA expression differed among the 3 muscles in the control group but not in the obesity group. The distribution of mRNA levels of MHC IId differed among the 3 muscles in the obesity group but not in the control group.

Conclusion: With a fatty diet, adipose accumulated, particularly in the genioglossus and geniohyoid muscles, and may influence the fiber type composition.

Basic studies of oral mucosal keratinocytes

1. Expression of the TSA α protein in oral epithelium

The multifunctional TSA α was originally described in T cells but is also expressed in epithelial cells from the respiratory tract and in endothelium. In this study, we found expression of TSA α mRNA and protein in both human and murine oral mucosal epithelium and in human primary oral keratinocyte cell cultures. In TSA α -/- mice, the mucosa and skin appeared macroscopically normal, but severe disturbances were observed in the fine structures of the basal membrane and intercellular epithelial spaces upon analysis with transmission electron microscopy. Oral epithelial cells from TSA α -/- mice displayed decreased migration compared with cells from wild-type mice, whereas overexpression of TSA α in a human epithelial cell line resulted in impaired proliferation. To our knowledge, this study is the first to show that TSA α is expressed in normal oral mucosa, is important for the normal ultrastructural morphology of the epithelium and basal membrane, and is involved in the migration and proliferation of oral keratinocytes.

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Institute of DNA Medicine

Department of Gene Therapy

Toya Ohashi, *Professor and Director*

Hiroshi Kobayashi, *Assistant Professor*

General Summary

Our purpose is to develop therapeutic methods for intractable diseases, including genetic diseases, cancer, and diabetes. We performed various studies and investigations this year. Below, we will describe the progress in each of our projects.

Research Activities

Genetics Disease

1. Development of gene therapy for lysosomal storage diseases:

We generated recombinant lentiviral vectors expressing missing enzymes in Krabbe disease and mucopolysaccharidosis type VII and administered these vectors to newborn model mice. For Krabbe disease, we detected increases in body weight but no effects on the progression of symptoms. For mucopolysaccharidosis type VII, effects on body weight and life span were observed, and lentiviral vector DNA copies were detected in organs, including the brain, of neonatal-treated model mice, findings that suggest efficient long-term expression.

2. Pathophysiological analysis of Pompe disease

We analyzed the relationship of autophagy with endoplasmic reticulum stress in fibroblasts derived from patients with late-onset Pompe disease. We found that autophagy is activated in patients' fibroblasts by mutant lysosomal acid/ α -1,4 glucosidase-induced endoplasmic reticulum stress and is inhibited by treatment with N-butyl-deoxynojirimycin.

3. Immune tolerance induction for enzyme replacement therapy for Pompe disease

Last year, we found that antibodies against infused enzymes in enzyme replacement therapy for Fabry disease decrease clinical efficacy. This year, we developed tolerance-induction therapy for enzyme replacement therapy for Pompe disease using a murine model. The oral administration of enzymes successfully induced immune tolerance. The formation of antibodies against enzymes is dependent upon T cells. Therefore, we administered anti-CD3 antibodies before enzyme infusion and successfully induced immune tolerance against the enzyme. Moreover, this tolerance persisted even if the enzyme was repeatedly infused. We are investigating the mechanism of this tolerance induction.

4. Bone marrow transplantation for lysosomal storage diseases

Although bone marrow transplantation is an effective treatment for lysosomal storage diseases, the minimum number of donor cells required to reduce storage materials remains unknown. To answer this question, we created a Fabry model mouse that carries various numbers of donor cells by the transplantation of various ratios of Fabry

mouse bone marrow cells and wild-type bone marrow cells to lethally irradiated Fabry mice. We found that 30% and 50% donor cells were enough to reduce storage material in the lungs and heart, respectively, and that these reduction values were equivalent to 100% donor cell reconstitution. Therefore, the intensity of the preconditioning regimen can be reduced, and this observation is beneficial for hematopoietic stem cell targeted gene therapy.

5. Antitumor effect and application to gene therapy of nafamostat mesilate for fatal digestive cancers

Recent studies have demonstrated that nuclear factor κ B (NF- κ B) plays an important role in the regulation of cell apoptosis, inflammation, and oncogenesis. Inhibition of NF- κ B is a potential new strategy for the treatment of cancer. We have shown that nafamostat mesilate, a serine-protease inhibitor used in Japan for the treatment of pancreatitis and disseminated intravascular coagulation and as an anticoagulant for hemodialysis, inhibits NF- κ B activation and induces apoptosis of pancreatic cancer. The combination of nafamostat mesilate and gemcitabine has been shown to be effective against pancreatic cancer in animal experiments and will be examined in clinical trials. We have also shown that the combination of paclitaxel and nafamostat mesilate is effective in models of peritoneal dissemination of pancreatic cancer; the combination is being studied in gastric cancer, which also tends to disseminate to the peritoneal cavity. Gene therapy with an adenoviral vector expressing tumor necrosis factor- α (TNF- α) is a new therapeutic approach for pancreatic cancer, for which treatment efficacy is limited, because TNF- α activates NF- κ B. We hypothesize that the addition of nafamostat mesilate, an NF- κ B inhibitor, may enhance the antitumor effect of adenovirus vector-mediated TNF- α gene therapy for pancreatic cancer.

6. Vitamin D receptor polymorphisms and the prognosis of patients with epithelial ovarian cancer

Recently, the vitamin D receptor (VDR) polymorphism FokI was shown to be associated with increased susceptibility to ovarian cancer. We examined whether VDR FokI polymorphisms affect the prognosis of patients with epithelial ovarian cancer. The VDR polymorphisms from FokI in 101 patients with epithelial ovarian cancer were genotyped by sequencing. Overall survival was compared between FokI single nucleotide polymorphisms by means of Kaplan-Meier survival analysis, log-rank tests, and the Cox proportional hazard model adjusted for International Federation of Gynecology and Obstetrics stages, postoperative chemotherapy, histologic type, and the presence of residual tumor. Hazard ratios, adjusted hazard ratios, and 95% confidence intervals were determined. The FokI C/C genotypes were associated with a better prognosis than were the C/T and T/T genotypes (log-rank test: $P=0.008$; adjusted hazard ratio: 0.16; 95% confidence interval: 0.05 to 0.57; $P=0.004$). Thirty months after surgery, 90% of patients with the FokI C/C genotype were still alive; in contrast, 66% of patients with the C/T or T/T genotype were alive. When the cancer stage of patients was restricted to II to IV, 84% of patients with the FokI C/C genotype were still alive: in contrast, only 50% of patients with the C/T or T/T genotype were alive. These results suggest that the VDR polymorphisms from the FokI genotype are associated with the improved prognosis of patients with epithelial ovarian cancer.

7. Molecular intervention therapy for pancreatic islets

- 1) We have continued studying molecular intervention therapy for pancreatic islets using an adeno-associated viral vector (serotype 8). We focused on possible mechanisms of beta cell death by islet injury, including oxidative stress, and planned to use several genes expressed in beta cells as therapeutic molecules.
- 2) To determine the mechanism of islet injury and its prevention, we have started to study the development of islet structures during murine embryogenesis. This year, we have investigated the interaction among islet endocrine cells and peri-islet Schwann cells.
- 3) We performed a comprehensive study of a novel system for delivering therapeutic molecules, such as insulin and glucose-like peptide 1. For this purpose, the steadiness of insulin injection with an ink-jet printer was studied by investigating glucose uptake into L cells and biochemical characteristics with high-performance liquid chromatography and matrix-assisted laser desorption ionization-time of flight mass spectrometer/spectrometry. We also investigated its bioavailability of the ink-jet insulin in vivo in rats.

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Institute of DNA Medicine

Department of Oncology

Mikio Zeniya, *Professor*
Junko Horiguchi-Yamada, *Associate Professor*

Sadamu Homma, *Associate Professor*
Shigeo Koido, *Associate Professor*

General Summary

The research goal of our department is to develop novel and modified therapeutic approaches for cancers and to explore new tumor markers indicating the presence of a cancer. We focused our efforts on studies of tumor immunology and cancer cell biology. Two clinical studies have been in progress to assess the therapeutic strategies in tumor immunology.

Research Activities

Mechanisms of synergistic effect of Wilms tumor 1 peptide vaccine and gemcitabine against pancreatic cancer

Clinical studies of Wilms tumor 1 (WT1) peptide and gemcitabine for the treatment of pancreatic cancer have achieved good responses. We investigated the basic mechanisms of these effects. Gemcitabine upregulates WT1 messages in human pancreatic cells through a nuclear factor kappa B pathway. The WT1 protein was shown to move from the nucleus to the cytoplasm. Furthermore, mass spectrometric measurement has shown an increase in the amount of WT1 peptide expressed on HLA-A*2402 molecules. These results indicate that gemcitabine-induced upregulation of WT1 can contribute to enhancement of immune responses.

Clinical immunotherapy for brain tumors

Treatment with the combination of fusion-cell therapy and temozolomide is in progress. Fusion cells were produced according to Good Manufacturing Practice procedures.

Crafting novel proteins composed of functional peptides for immunotherapy

To improve the efficiency of immunotherapy, we have started to develop artificial proteins to provide multiple candidates for antigens. As an initial trial, the proteins have been designed to contain WT1 peptide and α helix protein-stabilizing sequences, and the immunogenicity of these proteins is being examined. Once a library of designed proteins has been constructed and screened for immunogenicity, the association between the immune responses and physiochemical structure of the proteins can be studied.

Molecular mechanisms toward megakaryocytic differentiation induce by adhesion

A member of the ets transcription factor family, Friend leukemia virus integration 1 (FLI-1), has been investigated in human leukemia JAS-R cells, which can be induced to the megakaryocytic lineage by adhesion. Adhesion induces transcription of FLI-1, and

once this induction has occurred, the FLI-1 gene promoter is further activated through the positive feedback of FLI-1.

Confirmation of novel tumor markers for urological malignancies using tissue microarrays

Tissue microarrays were produced by rearranging paraffin-embedded blocks of cancerous and noncancerous tissue which were used as samples for histological studies. This system provides an efficient tool for studying possible tumor markers. Two candidates, Minichromosome maintenance 5 and glucosidase alpha neutral AB proteins, previously identified as tumor markers with proteomic methods, were confirmed with tissue microarray analysis to be upregulated in cancer tissues.

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Institute of DNA Medicine

Department of Molecular Genetics

Hisashi Yamada, *Professor and Director*

Takeshi Kawano, *Assistant Professor*

General Summary

With molecular genetics methods, the pathogenesis of many diseases has been revealed. To identify the genes responsible for each disease opens the door to the development of new treatments. The diseases that we are focusing on are malignant tumors, including hematological and pediatric malignancies. We are also investigating the molecular etiology of spinal muscular atrophy, Alzheimer's disease, and retinal diseases. Molecular pharmacological studies of anticancer agents are another part of our research. In particular, we are investigating histone deacetylase inhibitors (HDACIs), telomerase inhibitors, and DNA topoisomerase I inhibitors.

Research Activities

Exploring leukemogenesis

Leukemic stem cells (LSCs) and the structure of the hematopoietic stem cell niche have been vigorously studied in the past decade, and many discoveries have clarified how leukemic cells acquire resistance to intensive chemotherapy. We had initially thought that the characteristics of LSCs are similar to those of normal hematopoietic stem cells. It has become clear that leukemic cells have high plasticity. Therefore, general leukemic cells, on occasion, may de-differentiate to LSCs and may even transdifferentiate into mesenchymal stem cells.

We are attempting to confirm this plasticity of leukemic cells by using JAS-R megakaryocytic leukemia cells, which were established by us. We are now studying the plasticity of JAS-R cells by changing the culture conditions that mimic the microenvironment of bone marrow.

Molecular pharmacology of anticancer agents

Most cancers are managed with comprehensive therapeutic strategies. Radiation and chemotherapy are the main choices for medical oncologists to treat cancers. In our laboratory, we are studying the anticancer activities of telomerase inhibitors, HDACIs, and a DNA topoisomerase I inhibitors. In general, telomerase inhibitors express anticancer effects by inducing telomere shortening. For this reason, a long time is needed for a telomerase inhibitor to show anticancer effects. We used 5,10,15,20-tetrakis(*N*-methyl-4-pyridyl)porphyrin (TMPyP4) as a telomerase inhibitor. Unexpectedly, we found that TMPyP4 inhibited the telomerase activity but that its cytotoxic effects were observed within a few days without the shortening of telomeres. Instead, c-Myc was markedly downregulated in treated cells. Moreover, TMPyP4 directly induced DNA damage in treated cells. We propose that TMPyP4 has telomerase

inhibitory activity but can also induce apoptosis through the induction of DNA damage. We are also studying the anticancer effect of HDACIs. Our previous studies demonstrated that HDACIs are suitable drugs for combining with radiation. The dose of radiation to induce apoptosis of irradiated cells was reduced to 20% by simultaneous treatment with an HDACI. This augmentation was due to the stabilization of p53-tumor suppressor protein through the acetylation of p53 protein. This increased acetylation of p53 seems to interfere with the binding of p53 to an ubiquitin kinase.

Molecular genetical approach of neurological diseases

Spinal muscular atrophy is a degenerative disorder that leads to muscular atrophy. The mutation of survival of motor neuron (SMN) 1 is responsible for the onset of the disease. However, unlike other mammals, human beings have SMN2, a member of the same family as SMN1. We are studying why intact SMN2 cannot compensate for the function of SMN1 protein in patients with spinal muscular atrophy.

Alzheimer's disease (AD) is an incurable degenerative disease that ultimately leads to dementia. The signs and symptoms of AD are variable, and the clinical outcome of an individual patient is often difficult to predict at the onset of the disease. To evaluate the pathogenesis of AD more accurately, we are studying the relationship between the clinical subtypes and single nucleotide polymorphisms of brain-derived neurotrophic factor.

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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 has focused on the analysis of the basic immune system to protect against diseases and of immune disorders, such as hypersensitivity diseases and autoimmune diseases.

Research Activities

The roles of prostaglandin D₂ receptors chemoattractant receptor-homologous molecule expressed on helper T type 2 cells and D-prostanoid receptor in helper T type 1 and 2 cell inflammatory reactions

Prostaglandin (PG) D₂ is an essential modulator of inflammation that can both promote and alleviate inflammatory process. Many reports are consistent with the concept that the chemoattractant receptor-homologous molecule expressed on helper T (Th) type 2 cells (CRTH2) mediates the proinflammatory effects of PGD₂ in allergic inflammation. However, less clear is the role of the D-prostanoid receptor (DP) in inflammatory conditions. In this study, we investigated the roles of CRTH2 and DP in Th1 or Th2 inflammatory reactions *in vivo*.

Normal BALB/c mice and mutant mice lacking a functional CRTH2 or DP gene were used. To induce Th2-related inflammatory reactions, mice were infected with *Nippostrongylus brasiliensis* (*N. brasiliensis*), and serum IgE levels, the number of eosinophils, and production by spleen cells of antigen-specific Th2 cytokine were measured 2 weeks after infection. To induce Th1-related inflammatory reactions, mice were given subcutaneous injections of complete Freund's adjuvant, and interferon (IFN) γ production was measured for 72 hours in cultures of draining lymph node cells stimulated with purified protein derivatives. To analyze the role of CRTH2, ramatroban, a selective CRTH2 antagonist, was orally administered to some mice to inhibit CRTH2 signaling *in vivo*.

Allergic responses of IgE production, Th2 cytokine production, and eosinophilia caused by *N. brasiliensis* infection were reduced in CRTH2-knockout mice but were enhanced in DP-knockout mice compared with wild-type mice. On the other hand, complete Freund's adjuvant-mediated IFN- γ production was significantly enhanced in CRTH2-knockout mice but reduced in DP-knockout mice. Similar results were also observed in mice treated with ramatroban and in CRTH2-knockout mice. These results indicate the proinflammatory role of CRTH2 and the anti-inflammatory role of DP in Th2 inflammatory reactions, and, in contrast, the anti-inflammatory role of CRTH2 and proinflammatory role of DP in Th1 inflammatory reactions.

Staphylococcal enterotoxin B is involved in aggravation and recurrence of murine experimental autoimmune uveoretinitis via V β 8+ / CD4+ T cells

Endogenous uveitis is a common cause of visual disability and blindness. The etiology of uveitis remains unclear, but infection might be a cause. Superantigens are regarded as a leading cause of infectious etiology in autoimmune disease. However, the role of superantigens in uveitis remains unclear. In the present study, we investigated the effect of the superantigen Staphylococcal enterotoxin B (SEB) using an experimental model of autoimmune uveoretinitis.

C57BL/6 mice were immunized with human interphotoreceptor retinoid-binding protein (IRBP) peptide, and the severity of uveoretinitis was scored. Vehicle (phosphate-buffered saline [PBS]) alone or SEB dissolved in PBS was administered by intravenous injection on postimmunization day 10 or 24. In addition, a systemic immune response study was performed to address the effects of SEB on systemic immunity.

Uveoretinitis was aggravated significantly by injection of SEB on postimmunization day 10. Furthermore, relapse was induced by injection of SEB on day 24. On the other hand, SEB injection without IRBP peptide immunization elicited no inflammatory changes in the uvea or retina. Furthermore, SEB enhanced not only the IRBP-specific T-cell proliferative responses but also the production of IFN- γ and interleukin 17. Moreover, the intraocular expression levels of these cytokines was enhanced by SEB injection. Administration of antibodies against CD4 or V β 8 suppressed disease aggravation and the enhancement of IRBP-specific T-cell responses caused by SEB.

These results suggest that SEB is involved in the aggravation or recurrence of endogenous uveitis through activation of autoreactive uveitogenic T cells.

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Institute of DNA Medicine

Department of Molecular Cell Biology

Yoshinobu Manome, *Professor*

General Summary

Our research goals include molecular analysis and visualization of cellular events under both physiological and pathological conditions. To achieve our goals, we have used morphological and biochemical approaches combined with nucleic acid modification. We used transfection of DNA or short interfering RNA to modulate protein expression and fluorescent nanoparticles to visualize or quantify target molecules. Through the combination of molecular and cellular biology methods, we are exploring the medical life sciences.

Research Activities

Development of sonodynamic therapy and diagnostics for malignant glioma

Gliomas are common and intractable tumors of the brain. Because gliomas have an unfavorable prognosis, alternative therapies or treatments are needed. Despite the poor prognosis, distant metastasis is rare, and local recurrence is the most common cause of death. Therefore, long survival or even cure can be achieved only through the control of local recurrence. In this study, the prototype of a theragnosis system for glioma was produced. This system enables ultrasound to be applied to gliomas as a local treatment (therapy) while the process is monitored (diagnosis). The EUB6500 ultrasound diagnostic instrument (Hitachi Medical Corp., Tokyo, Japan) was used and combined with a therapeutic ultrasound generator and amplifier. The system can emit ultrasound at 500 kHz and 0 to 3 W/cm². The results demonstrated a beneficial effect of therapeutic insonation to gliomas in combination with microbubbles and suggested future clinical applications.

Three-dimensional cell culture of malignant glioma cells

Cell culture techniques are useful for observing the characteristics of tissues and organs in the human body. These techniques are also essential for the diagnosis and treatment of human disease. However, vital cell functions might be overlooked with ordinary 2-dimensional dish-based cell culture methods. For this reason, we used a culture method that mimics the human intracranial environment of gliomas. Structural changes have been observed in brain cell cultures using a gelatin scaffold as a bioabsorbable and biocompatible material. Because marked changes in morphological appearance can occur, we believe that signals or information of the cell membrane is important. Thus, expression of the transforming growth factor (TGF) β /SMAD system and matrix metalloproteinases (MMPs) was compared. The reverse transcriptase-polymerase chain reaction showed a difference in the expression of TGF- β , ALK5, ALK1, Smad 2, Smad

4, MMP-2, and p38 mitogen-activated protein kinase (MAPK). Among these, expression of genes ALK5, Smad 2, and Smad 4 differed considerably in 3-dimensional cultures. In contrast, differences in the expression of TGF- β , ALK5, MMP-2, and p38 MAPK were modest. Expression of these genes decreased in the 3-dimensional cell culture, and because the question of how the signal changes affect morphological differences in glioma needs to be answered, comprehensive analysis will be performed in combination with molecular targeting to the culture method. We also found that phosphorylation, but not the amount, of Akt significantly increased in the 3-dimensional culture. In addition, growth signals, such as platelet-derived growth factor subunit A of U118MG cells, which dispersed after cell division, increased more than 10 times compared with that of other cells, such as KNS42 and A172 forming agglomeration.

Detection of thyroid carcinoma antigen with quantum dots and JT 95 monoclonal antibody systems

Fluorescent nanoparticles, quantum dots (QDs), have been applied to biological studies and medical studies by taking advantage of their highly-intensity fluorescent properties. On the other hand, previous studies have shown the detection ability of the JT-95 IgM antibody with histological sections and the SW1736 thyroid carcinoma cell line. Therefore, we tested whether the combination of QDs and the JT-95 antibody could increase the ability to detect thyroid carcinoma. We showed that the combination of QDs and the JT-95 antibody could be used for biological analyses, such as Western blotting analysis and fluorescent microscopic analysis, of SW1736 cells. We have opened up the possibility that antibodies, even IgM, could be used to improve the ability to detect cancers.

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Institute of DNA Medicine

Project Laboratory for Kidney Regeneration

Takashi Yokoo, *Director*

General Summary

Many efforts are being made to apply regenerative medicine to the treatment of renal diseases. Renal diseases in which renal structure is maintained might be treated with infusions of stem cells isolated from the bone marrow or adult kidney. However such cell-based therapy cannot be used to treat chronic renal diseases, in which renal structures, including the kidney scaffold, are completely disrupted. Therefore, absolute kidney regeneration is needed to rebuild an entire functional kidney *de novo* and eliminate the need for dialysis. However, owing to the anatomical complexity of the kidney and the need for communication between cells to fulfill renal function, the kidney has been considered the most difficult organ to regenerate. Only a few groups are investigating the potential for reconstructing an organized and functional kidney structure, and, among them, we are using the developing xenoembryo as an organ factory for this purpose.

Research Activities

Use of the E2F1 transgenic suicide-inducible mice permits regeneration of complete human kidneys

We have established the transgenic estrogen receptor (ER)-E2F1 suicide-inducible mice that express the ER-E2F1 fusion protein. E2F1 is a transcription factor that regulates cell proliferation, and its ectopic expression induces apoptosis in differentiated cells; therefore, cells from ER-E2F1 mouse can be eliminated on demand by administering tamoxifen. Metanephroi from ER-E2F1 mice (E2F1 group) and C57BL/6 mice (control group) were cultured with or without tamoxifen for 14 days. The fate of cells was observed with fluorescence microscopy. Metanephroi were transplanted to Sprague-Dawley rats (N=10 in both groups), and FK506 and tamoxifen were administered daily for 2 weeks. The average weight of the grown transplants was recorded, and any fibrotic changes were observed histologically. The expression of erythropoietin was assayed with the quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) using species-specific primers.

Results: *In vitro* tamoxifen treatment of metanephros cells derived from ER-E2F1 mice successfully eliminated these cells, whereas wild-type cells remained viable. The percentage of the grown transplants *in vivo* was 32% in the E2F1 group versus 56% in control group ($P=0.045$). The final weight of transplants was 2.4 ± 0.8 mg in the E2F1 group and 8.7 ± 1.3 mg in the control group ($P=0.035$). Histological analysis showed that kidney tissue is replaced by fibrous tissue in the E2F1 group. Quantitative RT-PCR revealed that rat erythropoietin was produced from grown metanephroi in both

groups and that there was no significant difference between the groups.

Conclusion: Metanephroi derived from ER-E2F1 mice are a powerful tool to regenerate complete human-derived kidney tissue.

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Department of Neuroscience Division of Neuropathology

Satoshi Kurihara, *Professor and Director*
Junko Fujigasaki, *Assistant Professor*

Takahiro Fukuda, *Assistant Professor*

General Summary

Our research projects have concerned neurodegenerative disorders caused by intracellular accumulation of abnormal proteins. We are also studying mouse models of neurodegenerative disorders and autopsy cases by means of standard methods of morphologic analysis and molecular biological analysis.

Research Activities

Expression of hydroxyindole-O-methyltransferase enzyme in the human central nervous system and in pineal parenchymal cell tumors

Pineal parenchymal tumor (PPT) cells usually show immunoreactivity for synaptophysin, neuron-specific enolase, neurofilament protein, class III α -tubulin, tau protein, PGP9.5, chromogranin, serotonin, retinal S-antigen, and rhodopsin, but these markers are not specific for PPTs. Melatonin is produced and secreted mainly by pineal parenchymal cells; hydroxyindole-O-methyltransferase (HIOMT) catalyzes the final reaction in melatonin biosynthesis. We hypothesized that HIOMT could serve as a tumor marker of PPTs and investigated HIOMT localization and HIOMT expression in samples of normal human tissue and in PPTs, primitive neuroectodermal tumors, and medulloblastomas. In normal tissue, HIOMT was expressed in retinal cells, pineal parenchymal cells, neurons of the Edinger-Westphal nucleus, microglia, macrophages, thyroid follicular epithelium, principal and oxyphil cells of the parathyroid gland, adrenal cortical cells, hepatic parenchymal cells, renal tubule epithelium, and enteroendocrine cells of the stomach and duodenum. The expression of HIOMT was also found in all 46 PPTs examined. The proportions of HIOMT-immunoreactive cells successively decreased in the following tumors: pineocytoma, PPT of intermediate differentiation, and pineoblastoma. A few HIOMT-immunoreactive cells were observed in 1 of 6 primitive neuroectodermal tumors and in 23 of 42 medulloblastomas. These results indicate that immunohistochemical staining for HIOMT may be useful in the diagnosis of PPTs and be a prognostic factor in PPTs.

Neuropathology of Fabry knockout mice

Fabry disease is a rare X-linked recessive disorder caused by mutations in the alpha galactosidase A gene (GLA). As the result, globotriaosylceramide (GL3) and related glycosphingolipids accumulate in the lysosomes of many tissues and lead to organ failure. In this study, we investigated histopathological changes in GLA knockout mice and compared them with those in human Fabry disease. Male GLA knockout mice

were evaluated microscopically, ultramicroscopically, and immunohistochemically with an anti-GL3 antibody. GL3 accumulated in a variety of cells, including vascular endothelial cells, perithelial cells, and smooth muscle cells throughout the body. The storage material had a lamellar structure. Cells storing GL3 included proximal kidney tubule cells and macrophages and fibroblasts in a variety of organs. In the nervous system, GL3 accumulated in the cytoplasm of neurons in the trigeminal motor nuclei, solitary nuclei, spinal cord, trigeminal ganglions, dorsal root ganglia, and gastrointestinal tract. Electron microscopy revealed that the lamellar structure was present in axons and Schwann cells of peripheral nerves in the skin but not in the sciatic nerves. GL3 also accumulated in the cells of the pituitary gland, adrenal medulla, and spermatogenic cells. In a 67-year-old man with Fabry disease, GL3 immunoreactivity was observed in the neurons of the posterior nuclei of the hypothalamus and the dorsal motor nuclei of the vagus of the central nervous system and in a variety of cells, including cardiac muscle and renal glomeruli, of organs in which GL3 storage was not found in mice. We have demonstrated that GLA knockout mice show GL3 accumulation in the neurons and the axons of the nervous system, especially in the peripheral nerves of the skin.

Expression analysis of NCKX1 and phacr2 in a cellular model of spinocerebellar ataxia type 7

Spinocerebellar ataxia type 7 (SCA7) is a polyglutamine disease caused by polyglutamine expansion within a causative protein, ataxin-7. SCA7 is characterized by accentuated degeneration of the cerebellum and retina. Recent evidence suggests that ataxin-7 regulates transcription and that aberrant regulation of transcription is related to the pathogenesis of SCA7. We developed inducible PC12 cell lines expressing mutated/normal ataxin-7 (H111: ataxin-7-Q100, F127: ataxin-7-Q10). Expression array analysis using H111 demonstrated that about 300 genes decrease expression, with induction of mutated ataxin-7. Of these genes, we selected Na/Ca-K exchanger 1 (NCKX1) and phosphatase and actin regulator 2 (phacr2) and evaluated gene expression levels with the real-time reverse transcriptase-polymerase chain reaction. The expression of both genes decreased chronologically, only when mutated ataxin-7 was induced. NCKX1 regulates the intracellular calcium concentration of rod cells in the retina. Phacr2 is related to synapse plasticity in the Purkinje cells of the cerebellum. Suppression of the genes indicates that fluctuating expression of these genes could be related to the retinal and cerebellar degeneration in SCA7.

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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 are realized through intercommunication via the nervous system. To understand how the activities of organs affect brain activity and, in turn, how the brain controls the activities of the organs to optimize these integrative functions, it is absolutely necessary to clarify the mechanisms of dynamic cell-to-cell signaling in the central nervous system underlying various specific functions, such as autonomic regulation and pain sensation. In particular, plastic changes in central nervous system “wiring” realized through the variability of synaptic connections in response to various environmental changes are 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 and the real-time imaging of the intracellular Ca^{2+} concentration in living brain tissues from normal animals, animal models of various diseases, and animals with experimental manipulation of gene expression, and combine them with the behavior of these animals.

Research Activities

Central mechanisms of pain-related negative emotion

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

Glia-neuron interaction at central synapses

1. The role of monocarboxylate transport in synaptic function

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 postsynaptic responses in both the presence and absence of glucose supply.

2. RNA interference modulation of presynaptic ATP receptor properties

To clarify the roles played by specific molecules in transmitter release in brain synapses, we developed a novel method for *in-vivo* gene silencing with RNA interference against genes coding for presynaptic proteins. We succeeded in altering pharmacological characteristics of the presynaptic P2X receptors underlying extracellular ATP-evoked transmitter release, following reduced expression of subunit messenger RNA and protein by electroporation-mediated transfection of small interfering RNA into the nodose

ganglion.

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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 lower limbs and lower jaw. 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 Kyushu University, Osaka University, Tsurumi University, and the Mayo Clinic (Rochester, MN, USA).

Development of endoscopic surgical robot system

We are developing an endoscopic surgical robot system that can be used to perform natural orifice transluminal endoscopic surgery. Robotic instruments enter the abdominal cavity orally and are used to perform surgery on the abdominal organs.

This year, in addition to having the robot arm move freely (up and down, right and left, the opening and closing of the forceps), we added the function of having the robot arm rotate and move forward and backward. As a result, we constructed a prototype that can perform even more complicated surgery. By measuring the pulling force of the wires that drive the robot arms, we improved the functions that would display, to the operator, the softness of the objects that the robot arms grasp.

This research is being performed with Kyushu University's department of surgery.

Development of simulator for endoscopic surgical robot system

To perform surgery with the surgical robot system described above, the operator requires training because the operative method differs greatly from that of conventional surgery. Therefore, we are developing a simulator system for animal experiments that has the same functions as the actual surgical robot system.

This year, we constructed a prototype cockpit similar to that of the actual system and created an environment where the training could be performed with the same interface as the actual system. We could simulate surgical interruption of the inner membrane on a stomach lining model by coordinating the robot arm and a needle knife.

This research is being performed in collaboration with Kyushu University's department of surgery.

Development of an image-guided surgery system

We are developing a surgical navigation system that can perform data fusion for 3D images of the interior structures of veins, nerves, or tumors that cannot be seen with the naked eye when surgery is performed under the skin or within organs.

This year, in a collaborative study with our university's department of otorhinolaryngology, we performed stereoendoscopic sinus surgery 3 times and with our university's department of surgery performed hepatobiliary pancreatic surgery with laparotomy once in the high-tech navigation operating room of the Daisan Hospital.

Moreover, in the "intelligent surgical instruments project" being performed in collaboration with Kyushu University, we developed a data fusion display system that displays both the operative field and the navigational image, the patient's information during the operation, and a color-changing indicator to show the softness of objects the robot arm has grasped.

Application of the high-definition medical image analysis to forensic medicine

By applying technology that we have developed for analyzing high-definition medical images, we are analyzing X-ray CT data sets of crime victims with the aim of developing new methods for future criminal investigations and for establishing new methods for creating court documents.

This year, at the request of the Tokyo District Prosecutor's Office, we have analyzed the position, depth, and angle of a victim's wounds in 3D using the X-ray CT data set from a case of attempted murder.

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*

Akihito Tsubota, *Associate Professor*

Kouichi Nariai, *Assistant Professor*

Sadayori Hoshina, *Associate Professor and Deputy Director*

Yoshihisa Namiki, *Assistant Professor*

General Summary

This year is the 10th anniversary of the founding of the Institute of Clinical Medicine and Research in a corner of Kashiwa Hospital. Listed below are major works of the institute in clinical microbiology, liver disease and oxidative stress, the treatment of viral infections and cancer, the development of drug delivery systems, and lipid metabolism related to atherosclerosis.

Research Activities

Clinical microbiology

Blood stream infections and respiratory tract infections in children and adults with febrile neutropenia were studied with a magnetic filtration system for DNA/RNA extraction (Magtration, Precision System Science, Co., Ltd., Chiba, Japan), dual priming oligonucleotide polymerase chain reaction, and DNA microchip electrophoresis (MultiNA, Shimadzu Corp., Kyoto, Japan). Multiple infectious agents have been identified in both cases. Bioremediation of dioxins with thermophilic bacteria has been established for contaminated soil and has achieved reductions of 90% (W/W). Methods have been developed for treating infectious waste and chemical hazardous materials in hospitals and other institutions. Epidemiological studies were performed with the DNA diagnosis of pathogens.

Liver disease and oxidative stress

1. Gene expression profiling analysis for oxidative stress-induced liver carcinogenesis
Our group investigated how continuous exposure to reactive oxygen species produced in the oxidation-reduction reaction would affect carcinogenesis in chronic liver damage, using an animal model with naturally occurring and oxidative stress-induced hepatotumorigenesis. On the basis of our experiments, we have narrowed down numerous candidates to 2 signatures. Our gene expression profiling data have been uploaded to the National Center for Biotechnology Information's Gene Expression Omnibus Website.

2. Development of a novel antioxidant agent

We have verified the antioxidant activity of lactoferrin and proposed its mechanism of antioxidant action. We have completed a clinical randomized trial for chronic hepatitis C virus (HCV) infection and are analyzing treatment outcomes. We are planning to develop a new type of pegylated lactoferrin and to apply it to clinical practice.

Hepatitis C virus- and host-related factors associated with treatment outcome in pegylated interferon plus ribavirin combination therapy for chronic HCV infection

1. Analysis of factors predictive of treatment outcome in chronic HCV infection

To develop more rational and effective treatments for chronic HCV infection, we are improving antiviral treatment regimen and developing new agents by analyzing viral factors, such as kinetics and the HCV genome, and host-related factors, such as single nucleotide polymorphisms, in cooperation with the Division of Gastroenterology and Hepatology, Kashiwa Hospital.

Development of drug delivery systems

The aim of our research is to develop methods of anticancer drug release and magnetically guided nanostructures. To deliver sufficient amounts of therapeutic agents to deep internal tumor lesions with minimum drug doses, the combination of magnetic nanostructures and transplantable magnets are now being developed. We devised a new self-assembled nanoparticle formulation that can be magnetically delivered to silence genes in cells and tumor tissues (described in *Nature Nanotechnology* in 2009). This work has been supported by an Industrial Technology Research Grant, Program 08C46049a, in 2008 from the New Energy and Industrial Technology Development Organization of Japan, Grants-in-Aid for Scientific Research (B) from the Ministry of Education, Cultures, Sports, Science and Technology of Japan, the Life Science Foundation in 2009, the Futaba Electronics Memorial Foundation in 2008, the Takeda Science Foundation in 2007, and the Tsuchiya Foundation in 2006.

Studies of lipid metabolism and atherosclerosis

1. We studied the effects of carbohydrate feeding on postprandial hyperlipidemia by measuring serum levels of apolipoprotein B-48.

2. We are performing an incubation study using bacteriophages to examine the antiviral effects of plasma fractions. This work will investigate the connection between lipid metabolism and susceptibility to viral infection.

3. With our newly developed method of high-performance liquid chromatography (reported in *Clinical Biochemistry* in 2007 and in *Lipids in Health and Disease* in 2008) we established a method for measuring lipoprotein A, and a manuscript presenting this method was accepted for publication in the *Journal of Lipid Research*.

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Medical Engineering Laboratory

Hiroshi Furuhashi, *Professor*

Masayuki Yokoyama, *Associate Professor and Director*

Koichi Kanemoto, *Associate Professor*

General Summary

The Medical Engineering Laboratory has developed new ultrasonic therapeutic technologies. This year, our safety study of sonothrombolysis was performed with various animal models of risk factors for acute stroke. Basic research studies in molecular medical engineering to develop an ultrasonic drug delivery system have also been continued. In particular, the safety of nanodroplets, whose phase transition from fluid to gas is caused by ultrasonication, has been evaluated histopathologically and immunochemically. *In vivo* ultrasonically mediated nitric oxide generation has been measured with a real-time monitoring system.

We developed a nanocarrier system with novel polymer chemistry for the delivery of anticancer drugs and diagnostic imaging agents. A current topic is a immunity to nanocarriers, such as polymeric micelles and liposomes, by repeated injection. Measurement of anticancer drugs encapsulated in polymeric micelles by means of synchrotron small-angle X-ray scattering (SAXS) was performed. We obtained the inner core size, outer shell length, and drug distribution in the core of polymeric micelles.

Research Activities

Study of a special consortium for supporting the development of cutting-edge medical care (Ministry of Health, Labour and Welfare)

Safety studies of our new method of transcranial sonothrombolysis have shown no increase in the hemorrhage rate or in ischemic lesions, compared with a thrombolytic agent alone, for the treatment of high-risk animals with hypertension, severe arteriosclerosis, and previous stroke.

A magnetic resonance imaging (MRI) system with a high spatial resolution (9.4 T) was set up at the experimental animal facility.

The safety of an ultrasonic system with nanodroplets

This novel ultrasonic system with nanodroplets uses the heating effect caused by bubble cavitation. The histopathological safety index of ultrasound can be exploited for various ultrasonic therapies.

Nitric oxide generation by ultrasonic stimulation

Transcutaneous ultrasonic mediation caused nitric oxide generation in an RT-2 glioma-bearing mice.

Relation of polymeric micelle MRI contrast agent to the accelerated blood clearance phenomenon

PEGylation, the attachment of polyethyleneglycol (PEG), to nanoparticles can enhance the duration of their circulation in the bloodstream. However, repeated injection of PEGylated liposomes induces the accelerated blood clearance (ABC) phenomenon. We investigated the ABC phenomenon for a gadolinium-containing polymeric micelle (Gd-micelle), which is formed by the hydrophilic interaction, and for a gadolinium-containing PEGylated liposome (Gd-liposome) in mice.

The effects of the first injection of Gd-micelles on the tissue distribution of the second dose of Gd-micelle were examined. In addition, the effects of the first injection of Gd-micelle, Gd-liposome, and PEGylated liposome on the distribution of the second dose of the Gd-liposome were evaluated. The results indicate that the tissue distribution of the second injection of the Gd-micelle was not affected by the first injection of the Gd-micelle at different doses and time intervals. On the other hand, the first injection of the Gd-liposome or the PEGylated liposome induced the ABC phenomenon. The induction mechanism of the ABC phenomenon is suggested to be the production of anti-PEG IgM after the first injection of the PEGylated liposome. However, in the case of our polymeric micelle with PEG chain, we observed no induction of the ABC phenomenon with the first injection. This result indicates that our polymeric micelle MRI contrast agent does not induce such immunity when used as a diagnostic agent.

Exploration of hydrophobic drug encapsulated polymeric micelle core

SAXS was performed with aqueous polymeric micelle solutions at the 40B2 and 45XU beamlines of the Super Photon Ring 8 GeV (Large-scale Synchrotron Radiation Facility). The block copolymer consists of PEG and partially benzyl-esterified poly(aspartic acid) (P(Asp(Bzl))). The block copolymer encapsulated a hydrophobic model drug, such as LE540, which is an antagonist of the retinoic acid receptor. The scattering profiles of the polymeric micelle-encapsulated LE540 showed characteristic features for core-shell spherical micelles, confirming that P(Asp(Bzl)) forms a hydrophobic core and that PEG forms a hydrophilic shell. When the loading amount of LE540 is increased, the core structure of the polymeric micelles is markedly changed. The SAXS data indicated that when LE540 is added, the core radius increases from 5.9 to 6.9 nm, but the shell radius remains at 12.5 to 12.8 nm. We concluded that LE540 stably encapsulated in polymeric micelles is homogeneically distributed in the core.

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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 pilot study in 3 major hospitals, including our hospital, according to a case-cohort study design in which detailed data were collected in all cases and in a subcohort representing 5% of the whole sample. A full-scale study has been performed with a large sample size of 20,000 patients.

An administrative office for registration trials was established in the hospital in February 1999, and the system for registration trials in the hospital has been reformed to meet the demands of the new good clinical practice (GCP) guidelines. Seven clinical research coordinators (CRCs) — 6 nurses and 1 pharmacist — now facilitate clinical trials. The CRCs have started to help with both registration trials and investigator-initiated trials. CRCs 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 done abroad. This action plan selects 10 core hospitals and 30 major clinical trial institutions. The Jikei University Hospital applied to be a major clinical trial institution and was accepted. According to this plan, we reinforced CRCs and introduced a data manager to improve the clinical trial system. We also introduced an information technology system to processing registration trial management.

Publications

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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 2009 through March 2010, we held 10 seminars about strategies for clinical studies for healthcare practitioners at The Jikei University. In 2009, 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. New drug development related to vitamin D
3. Epigenome clinical studies and lead findings

Publications

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Division of Clinical Epidemiology

Masato Matsushima, *Associate Professor and Director*

General Summary

Division of Clinical Epidemiology was established in 2009 as a part of Research Center for Medical Science to promote clinical research, clinical epidemiology, and education concerning them. Our aim is to support clinicians solving problems in daily practice through epidemiological and clinical research skills.

Our research areas, moreover, cover medical communication, evaluation of medical care, behavioral medicine, outcome research, qualitative research, and disease-oriented epidemiological research. Especially, we aim to address the lack of evidence in the field of primary care.

As a contribution to undergraduate education, our division gives a class called “Evidence-Based Clinical Practice (EBCP)” to help medical students become skillful physicians who can use an evidence-based approach.

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

Research Activities

Historical cohort study of fever and infection in a setting of home-medical care

This is the first attempt to evaluate the incidence of fever in a Japanese home-medical care setting. The number of participants was 105 (M : F 37 : 68) with the mean (\pm SD) age of 82.8 ± 7.9 years in one primary-care clinic. The total of person-days were 27,546. The incidence of fever $> 37.2^{\circ}\text{C}$ was 2.32/1,000 person-days (95%CI: 1.75–2.89). Approximately half of the individuals receiving home-medical care experienced fever once a year.

Chronic care model

Chronic care model was developed in the 1990's in the United States to improve chronic illness care by refining the care-provider system especially in the primary-care setting. The aim of this research is to clarify the usefulness of the chronic care model in Japan. The plan consists of three steps. The first is to create the official Japanese version of the assessment form “Assessment of Chronic Illness Care (ACIC)” by following the World Health Organization procedure, for instance, translation, back translation, and pilot study. This procedure has been completed. The second step will be to compare the quality of diabetes care between specialists in diabetes and primary-care physicians as

non-specialists. The last step will be to perform a cluster randomized controlled trial to evaluate the efficiency of “Chronic care model”.

The effect of a communication style in medical interviews on patients' satisfaction

At present, the patient-physician relationship is of great interest in Japan. However, few studies have been reported. This study evaluated the effect of a communication style in medical interview on patients' satisfaction with the Roter Method of Interaction Analysis System (RIAS).

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Laboratory Animal Facilities

Kiyoshi Ohkawa, *Professor and Director*

Koichi Nariai, *Assistant Professor*

General Summary

The purpose of the Laboratory Animal Facilities (LAF) is to support *in vivo* research and to contribute to the development of basic and clinical medicine. In 2009, 176 researchers used the LAF. We undertake breeding of experimental animals and guide researchers in the techniques of animal experimentation. In addition, we performed the following studies to develop basic medical sciences, including laboratory animal science.

Research Activities

Ovulation inhibition due to removal of peripheral blood phagocytes

Reactive oxygen species (ROS) containing superoxide are believed to be involved in ovulation. By using a specific superoxide sensor we have recently confirmed the production of superoxide and showed the immunohistochemical localization of DNA and lipid peroxides in the ovulating ovary. Phagocytes, such as neutrophils and macrophages, are thought to be the sources of the ROS involved in ovulation. We examined whether the removal of peripheral blood phagocytes inhibits ovulation, to examine the source of the ROS involved in ovulation.

Induction of follicular regression by photodynamic therapy

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

*Establishment and characterization of the strains originated from Japanese wild mice (*Mus musculus molossinus*) and *Phodopus* hamster*

Inbred strains that we derived from Japanese wild mice (*Mus musculus molossinus*) and *Phodopus* hamsters were maintained in this laboratory.

Japanese wild mice are classified as *M. m. molossinus* and originated from a natural intersubspecific hybrid between *Mus musculus castaneus* inhabiting southwest Asia and *Mus musculus musculus* distributed in north Asia. The *M. m. molossinus* subspecies is an excellent material to improve laboratory mice, because its gene constitution is suspected to differ greatly from that of the common laboratory mice derived from the *Mus musculus domesticus* subspecies. We have established several new inbred strains based on *M. m. molossinus* mice captured in Osaka prefecture. These strains are being

maintained in our laboratory, and new consomic strains based on these strains are being developed.

In collaboration with the Department of Molecular Biology, we developed 2 new mouse strains using a *M. m. molossinus* inbred strain we developed and named MSKR. One is a congenic strain having a knockout allele of *Oaz1* derived from the B6.129-*Oaz1*tm to the MSKR background, and the other is a consomic strain that has chromosome 10 derived from the above-mentioned strain to the MSKR background. We have confirmed that these newly established strains are useful for research into the genetic modification of *Oaz1* knockout mice.

Phodopus hamsters are small rodents that differ taxonomically from Syrian hamsters, which are the most commonly used laboratory hamsters. We recently determined that the *Phodopus* hamster is a good candidate for a new laboratory animal and have established an inbred strain. Furthermore, we continue to establish other inbred strains and congenic strains, to develop human disease models, and to research its biomedical characteristics.

The search of the novel atopic dermatitis therapeutic drug using NC/Nga inbred strain

The NC/Nga inbred strain is the current mouse model for atopic dermatitis. However, the onset rates of dermatitis differ among lines maintained at different laboratories. The NC/Nga inbred strain maintained in our laboratory is a line with a particularly severe dermatitis diathesis.

In collaboration with the Department of Tropical Medicine, we are using NC/Nga mice to search for new drugs for treating atopic dermatitis.

Publications

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Radioisotope Research Facility

Kunihiko Fukuda, *Professor and Director*

Yukio Yoshizawa, *Assistant Professor*

General Summary

The Radioisotope Research Facility was established to support medical and biological research with isotopes. We have supported researchers by suggesting methods and practical techniques for experiments. Lectures and training sessions were held for researchers and for medical students and graduate students who are starting to work with radioisotopes. In 2009, 44 researchers from 12 departments used the laboratory in this facility. Major nuclides used for experiments were ^{32}P , ^{51}Cr , ^{125}I , ^{35}S , and ^3H .

Research Activities

The active site of exfoliative toxin A

Exfoliative toxins A and B (ETA and ETB) produced by *Staphylococcus aureus* cause staphylococcal scalded-skin syndrome. We have previously reported that the nitration of tyrosine residues causes both ETA and ETB to lose all toxicity. Site-directed mutagenesis was used to introduce mutations at either tyrosine (Tyr)-17-18 or Tyr-225-232. Substitution of any Tyr residues with phenylalanine destroyed ETA activity. The agglutination titer of mutated ETAs was $1/40^{\text{th}}$ that of native ETA. On immunodiffusion test, substitution of Tyr-17-18 or Tyr-225-232 resulted in a complete loss of immunoreactivity with anti-ETA rabbit serum, thus confirming that Tyr-17-18 and Tyr-225-232 are essential for the toxicity and antigenicity of ETA.

Development of techniques for determining radioactivity

Radon is a radioactive noble gas produced by the decay of radium present in soil and rocks. Radon is the second most common cause of lung cancer in the general population, after smoking. A reference level of 300 Bq/m^3 was proposed by the International Commission on Radiological Protection to minimize the health hazard due to indoor radon exposure. We have used silicone oil as a liquid scintillation solvent to measure radon indoors. The silicone oil is transparent, nonvolatile, physiologically inert, and heat-resistant. These properties are thought to be suitable for a scintillator solvent. Methylphenyl silicone HIVAC F-4 (Shin-etsu Chemical Co., Ltd., Tokyo, Japan) is an excellent liquid scintillation solvent for radon determination.

Study of resistance mechanisms in radiation-resistant organisms

Tardigrades show remarkable adaptability in extreme environmental conditions, such as high radiation. To study the mechanism of radiation resistance in organisms, we examined the effect of radiation on tardigrades. Tardigrades were collected from moss on the streets around The Jikei University and activated sludge from the Ariake Water Reclamation Center (Bureau of Sewerage, Tokyo Metropolitan Government). Tardi-

grades isolated from moss were identified by 18S-rDNA as *Microbiotus* and *Milnesium tardigradum*, and those isolated from activated sludge were identified as *Isohypsibius*. Because the drying tolerance of *Isohypsibius* differs from that of other tardigrades, we believe that comparing the effects of radiation on them with drying tolerance tardigrades will be worthwhile.

Determination of trace elements in cosmetics by radiochemical methods

There are many inexpensive cosmetics on the market whose adverse effects on health are frequently overlooked. Colorful cosmetics, particularly for eye makeup, often contain toxic heavy metals as pigments. To determine what metallic elements are present in cosmetics and to estimate their toxicity, eye cosmetics were analyzed with instrumental neutron activation analysis. The concentrations of Mg, Al, Sc, Ti, V, Cr, Mn, Fe, Co, Zn, Cs, and Ta in 30 eye shadows were determined. From cross-correlation of elemental abundances and the relationship with their color, we estimated the origin of the elements.

Publications

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Core Research Facilities

Yoshinobu Manome, *Professor and Director*
Takeo Iwamoto, *Associate Professor*

Hiroyuki Sasaki, *Associate Professor*

General Summary

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

Annual Registration System

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

System for Providing Research Services

Advances in research technologies and equipment enable us to perform more precise and accurate observations of specimens in medical sciences. However, the high technology and various new 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 experimental. 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

Biotracing using fluorescent nanoparticles

The monoclonal antibody JT95 was developed at this university. It specifically recognizes an antigen expressed in differentiated thyroid carcinomas. For the use of serum in the diagnosis of thyroid cancer, the antibody was conjugated to fluorescent nanoparticles. The localization of the antigen was visualized with fluorescence microscopy after

reactions with thyroid carcinoma cells. In addition, the conjugate demonstrated greater sensitivity than the enzyme-linked immunosorbent assay for quantitative analysis of the antigen in human serum.

Functional analysis of tight junctions

Tight junctions (TJs) in epithelia and endothelia restrict the paracellular flux of water and solutes. Epidermal TJs are thought to restrict molecular movement and to help the stratum corneum serve as a secondary barrier in the skin. Calcium ion (Ca^{2+}), a well-known differentiation inducer for keratinocytes, distributes to form a vertical gradient peaking in the stratum granulosum. In this study, we applied sodium caprate, which elicits dilations of TJs on human reconstructed epidermis, and investigated Ca^{2+} distribution in the epidermis. Ion-capture cytochemistry and electron energy-loss spectroscopy revealed that treatment with sodium caprate markedly altered Ca^{2+} localization in the epidermis. Additionally, abnormal differentiation (e.g., parakeratosis) was observed in the stratum granulosum. To confirm that these changes were caused by TJ disruption, we observed the structure of TJ strands with the freeze-fracture replica method and measured transepidermal Ca^{2+} permeability by quantifying diffused Ca^{2+} through the epidermis. We found that the TJ strands had fragmented and that Ca^{2+} permeability had increased. These data suggest that epidermal TJs maintain Ca^{2+} under the stratum corneum and regulate epidermal differentiation.

A channel-forming peptide that modulates drug delivery across in vitro corneal epithelium

The goal of this study was to determine whether a synthetic peptide, NC-1059, can modulate the corneal epithelium to increase the permeation of therapeutic agents across this barrier. An *in vitro* system employing transformed human corneal epithelial cells was optimized for this study. Culture conditions were identified to promote the formation of a confluent monolayer that rapidly develops a substantial transepithelial electrical resistance. Electrical parameters were measured with a modified Ussing flux chamber, and solute flux was quantified with fluorescently labeled compounds. The peptide NC-1059 causes a concentration-dependent increase in short-circuit current and an increase in transepithelial electrical conductance when assessed in a modified Ussing chamber. The effect of NC-1059 on transepithelial electrical resistance was reversible. To test for paracellular permeability and size exclusion, fluorescein isothiocyanate-labeled dextran ranging in size from 10 to 70 kDa was used. Dextran permeated the corneal cell monolayer in the presence, but not the absence, of NC-1059.

When fluorescein sodium and carboxyfluorescein were used as low molecular weight markers, similar NC-1059-modulated kinetics were observed.

Maximum permeation for the fluorescein derivatives occurred 30 to 90 minutes after exposure to NC-1059 for 5 minutes.

A prototypical drug, methotrexate, also exhibited increased permeation in the presence of NC-1059. NC-1059 enhances drug permeation across cultured corneal epithelial cell monolayers by transiently affecting the paracellular pathway. Thus, NC-1059 is a lead compound for the development of cotherapeutic agents to enhance the access and

effectiveness of ophthalmic compounds.

Publications

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Department of Genetic Disease Research (Lysosomal Storage Disease)

Yoshikatsu Eto, *Professor and Director*
Takashi Higuchi, *Postdoctoral Fellow*

Collaborating Researcher
Hiroyuki Ida, *Professor*
Toya Ohashi, *Professor Collaborating Researcher*
Hiroshi Kobayashi, *Assistant Professor Collaborating Researcher*
Masayuki Kobayashi, *Assistant Professor Collaborating Researcher*

General Summary

The Donated Department of Genetics and Genome Science was established on April 1, 2008. The main topic studied is the basic pathogenesis of genetic diseases, particularly, lysosomal storage diseases (LSDs). In particular, the pathogenesis of central nervous system (CNS) involvement in LSDs is the most important problem. To understand pathophysiology of the CNS events, we have generated induced pluripotent stem cells (iPSCs) from mucopolysaccharidosis (MPS) VII mice and caused them to differentiate into neuronal cells. We also generated iPSCs from model mice of Fabry disease and Pompe disease and caused them to differentiate into cardiac cells and skeletal muscle cells. Furthermore, we attempted to treat CNS involvement of LSDs by intrathecal injection of enzymes into MPS II mice. The promising results suggest that lysosomal storage in neuronal cells can be treated in MPS II mice.

Research Activities

1. To establish novel treatments for CNS involvement is an intriguing problem; such treatments include the intrathecal or intraparenchymal injection of enzyme into MPS II mice. We also performed gene therapy of Pompe disease by means of a lentivirus vector. Sufficient expression of the alpha-glucosidase gene was observed in cardiac muscle, but expression was less in skeletal muscle.
2. To establish new technologies of iPSCs from various LSDs for understanding the pathophysiology of LSDs and developing new therapies, we successfully isolated iPSCs from skin fibroblasts of twitcher, Fabry, and Sly mice and caused them to differentiate into many cell types. Four factors were used — hKlf4, hSox2, hc-Myc, and hOct — and Myc was also deleted to isolate iPSCs (Mao, Shen, Ohashi, Eto, 2009). We also recently established the iPSCs from human Pompe disease and caused them to differentiate into skeletal muscle cells.
3. We evaluated the efficacy of enzyme replacement therapy in terms of serum antibody titers in patients with Fabry disease and Pompe disease. Results indicated that high antibody titers in serum inhibited enzyme uptake and neutralized activities. These results indicate that high serum antibody titers against enzymes influence the efficacy of enzyme administration for patients with Fabry disease and Pompe disease.

4. To establish new screening procedures for LSDs with dried blood spots is an important technology for the early diagnosis and treatment of patients with Pompe disease and Fabry diseases. We established the dried blood spot method for the early diagnosis of Pompe disease and Fabry disease and other LSDs using 4-methylumbelliferyl derivatives.

Publications

Okuyama T, Tanaka A, Suzuki Y, Ida H, Tanaka T, Cox GF, Eto Y, Oritani T. Japan Elaprase((R)) Treatment (JET) study: Idursulfase enzyme replacement therapy in adult patients with attenuated Hunter syndrome (Mucopolysaccharidosis II, MPS II). *Mol Genet Metab* 2009; 18-25.

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

Sakurai Y, Kojima H, Shiwa M, Ohashi T, Eto Y, Moriyama H. The hearing status in 12 female and 15 male Japanese Fabry patients. *Auris Nasus Larynx* 2009; **36**: 627-32.

Review and Books

Eto Y. Pompe disease; History in Book "Pompe Disease" (Japanese). Tokyo: Shindanto Chiryousha; 2009. p. 2-8.

Eto Y. Fabry disease Guideline of Diagnosis and Treatment of Fabry Disease (in Japanese). Shinkei Shitsukan Guideline. Tokyo: Sougou-Igakusha; 2009. p. 178-80.

Eto Y. Congenital enlargement of kidney-Fabry disease. *Kidney Dial* 2009; **66**: 161-4.

Eto Y. Inherited lipid metabolic disorders (in Japanese). *Nihon Rinsho* 2009; **9**: 663-79.

Eto Y. Enzyme replacement therapy of Fabry disease. EBM based Treatment for Neurological disorders (in Japanese). *Chiryō* 2009; 360-6.

Presentations

Kyosen SO, Iizuka S, Kimura T, Kobayashi H, Fukuda T, Ida H, Eto Y, Ohashi T. Neonatal gene therapy using lentiviral vector system for murine Pompe disease: long term efficacy. Japan Society of Gene Therapy. The 15th annual meeting. Osaka, 2009. July 9-11.

Eto Y. The 21 millennium Goal for Child Health & Developmental Medical Science: Current Status and Future Prospects of Child & Adolescent Medicine in Japan. 5th Congress of Asian Society for Pediatric Research (ASPR). Zhijiang China. 2009, May 21-23.

Eto Y. Recent Advances of the Treatments and their Problems in Lysosomal Storage Diseases (LSD). The 3rd International Symposium of Lysosomal Storage Disease/The 14th Japanese

Society for Lysosomal Storage Disease. Nagoya, 2009. September 26-27.

Ohashi T, Iizuka S, Kobayashi H, Shimada Y, Eto Y, Ida H. Immune tolerance induction in enzyme replacement therapy for Pompe disease by anti-CD3 antibody and oral enzyme administration. The 3rd International Symposium of Lysosomal Storage Disease/The 14th Japanese Society for Lysosomal Storage Disease. Nagoya, 2009. September 26-27.

Ishige N, Suzuki K, Ohashi T, Eto Y, Owada M, Kitagawa T. Reevaluation of usefulness of urinary GL-3/creatinine concentration for diagnosis of Fabry disease. The 3rd International Symposium of Lysosomal Storage Disease/The 14th Japanese Society for Lysosomal Storage Disease. Nagoya, 2009. September 26-27.

Shimada T, Yokoi T, Kobayashi H, Eto Y, Ohashi T. Characterization of endoplasmic reticulum stress response in Pompe disease. The 3rd International Symposium of Lysosomal Storage Disease/The 14th Japanese Society for Lysosomal Storage Disease. Nagoya, 2009. September 26-27.

Shimizu H, Kobayashi H, Toya O, Hiroyuki I, Kawai M, Eto Y. High-risk screening of Pompe disease using DBS in muscular dystrophy hospitals. The 3rd International Symposium of Lysosomal Storage Disease/The 14th Japanese Society for Lysosomal Storage Disease. Nagoya, 2009. September 26-27.

Okuyama T, Tanaka A, Suzuki Y, Ida H, Tanaka T, Eto Y, Oritani T. Japan Elaprase Treatment (JET) Study: Idursulfatase Enzyme Replacement Therapy in Adult Patients with Attenuated Hunter Syndrome. The 3rd International Symposium of Lysosomal Storage Disease/The 14th Japanese Society for Lysosomal Storage Disease. Nagoya, 2009. September 26-27.

Ohashi T, Iizuka S, Eto Y, Ida H. Impact of Antibody Formation for Enzyme Replacement Therapy for Lysosomal Storage Diseases and Immune Tolerance Induction for Infused Enzyme. The 11th International Congress on Inborn Errors of Metabolism. San Diego, USA, 2009, August 29-September 2.

Meng XL, Shen JS, Kawagoe S, Ohashi T, Eto Y. Implications of Induced Pluripotent Stem (IPS) Cells on Mechanistic Study for Lysosomal Storage Diseases. The 11th International Congress on Inborn Errors of Metabolism. San Diego, USA, 2009, August 29-September 2.

Kobayashi H, Shimada Y, Fujigasaki J, Ariga M,

Ohashi T, Eto Y, Ida H. Pathology and mechanism including autophagy in the autopsy case diagnosed as mucopolipidosis type III. The 11th International Congress on Inborn Errors of Metabolism. San Diego, USA 2009, August 29-September 2.

Okuyama T, Tanaka A, Tanaka T, Ida H, Suzuki Y, Cox GF, Eto Y, Torii T. Idursulfase enzyme replacement therapy in seriously ill, Japanese Men with Hunter syndrome. The 11th International Congress on Inborn Errors of Metabolism. San Diego, USA, 2009, August 29-

September 2.

Eto Y. Novel Treatments and their Problems in Lysosomal Storage Disease (LSD). 13th Asian Pacific Congress of pediatrics and 3rd Asian Pacific Congress of Pediatric Nursing. Shanghai, China, 2009, October 14-18.

Eto Y. Recent Advances of Treatment for Genetic Disease. 13th Asian Pacific Congress of pediatrics and 3rd Asian Pacific Congress of Pediatric Nursing. Shanghai, China, 2009, October 14-18.

Department of Rehabilitation Medicine

Division of Physical Fitness

Masahiro Abo, *Professor and Director*

Hideki Yamauchi, *Assistant Professor*

General Summary

The research of our division has been focused on skeletal muscle plasticity, neuroscience, and exercise physiology.

Research Activities

The availability of heat stress is reported in the regeneration of damaged skeletal muscle. We examined mechanisms underlying the regeneration-promoting effect of heat stress (warm bath of 42°C for 30 minutes). Heat stress before damage accelerated the regeneration of myofiber size 6 days after the damage. We found HSP72 expression and Akt activity tended to be higher 6 days after damage. Therefore, activation of Akt and higher expression levels of stress proteins may be associated with the promotion by heat stress of muscle fiber regeneration.

We studied muscle fiber atrophy and regeneration with hindlimb unloading in adulthood and old age. Muscle atrophy due to unloading was greater in old age than in adulthood. We found deranged intramuscular structures and inclusion bodies with unloading in type I-predominant regions in both age groups. Although we observed muscular fibers with vacuoles of tubular aggregates in type II-predominant regions, denaturation was less than in type I-predominant regions. Such degeneration was more marked in old age than in adulthood.

Myostatin, a member of the transforming growth factor β superfamily, is a negative regulator of myogenesis and muscle hypertrophy. We examined the effects of hindlimb unloading with or without intermittent reloading on soleus muscle mass and expression levels of myostatin protein in rat skeletal muscles. We found that myostatin protein levels increased with hindlimb unloading. Intermittent reloading restrained expression changes. We conclude that myostatin plays important roles in the regulation of muscle mass.

Numerous studies have examined changes in blood adipocytokine density after exercise and diet therapy, but less is known about the rebound period after weight reduction. We examined adipocyte size and blood adipocytokine density immediately after and 2 days after exercise and diet therapy in Otsuka Long-Evans Tokushima fatty rats. Our findings suggest that blood leptin density in the rebound period may change independently of fat volume or the adipocyte size.

Exercise that is prolonged or of high intensity or both suppresses immune responses. However, the relationship between exercise intensity and the suppression of lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF)- α is not fully understood. We studied the effects of different exercise intensities on LPS-induced TNF- α production in

rats. We concluded that the suppression of LPS-induced TNF- α production depends on exercise intensity in rats.

Publications

Kitamura H¹, Minato K¹, Kimura M², Yamauchi H, Yano H³ (¹Wayo Women's Univ, ²Keio Univ, ³Kawasaki Welfare Univ). Lipopolysaccharide-

induced tumor necrosis factor (TNF)- α production depends on exercise intensity in rats. *Jpn J Phys Fitness Sports Med* 2009; **58**: 405-8.

Department of Cell Physiology Division of Aerospace Medicine

Satoshi Kurihara, *Professor*
Hiroko Toshima, *Assistant Professor*

Masamichi Sudoh, *Associate Professor*

General Summary

Our main research interests are gravitational physiology and aerospace medicine. We also developed a new method with which human mental concentration and relaxation can be objectively evaluated.

Research Activities

Research on visual stimulus and posture control

The information used to maintain body direction and to produce movements of the body center to maintain posture are determined by visual input factors, equilibrium vestibular input factors, and somatosensory factors from the muscles, tendons, joints, and skin of the entire body. Visual information becomes the main factor in outer space because the vestibular and somatosensory inputs are reduced owing to the lack of gravity. The aim of this research was to analyze the change of posture induced by visual stimulus. This year, we developed the following device: a roll screen 90 cm in diameter and 110 cm high which has vertical black and white 7-cm-wide stripes on its inner surface and can rotate at speeds of 5, 10, and 20 rpm. The center of gravity of the body of each subject was measured with a balance board (a modified Wii Balance Board, Nintendo Co., Ltd., Kyoto) that was placed at the center of the roll screen. The data of the balance board was sent to a computer via a data acquisition system (PowerLab, ADInstruments Japan, Tokyo) on line. First, the subjects stood on the board and then watched the rotating screen (visual stimulus). Next, the subjects stepped on the board with or without visual stimulus.

Changes in the body's center of gravity in the static standing position were observed when the screen was rotated, but there was no significant difference between with and without the visual stimulus. However, a significant rotational change of the body was observed when the subjects stepped on the board. The rotation angle showed individual patterns, but the direction of rotation was the same as that of the screen.

Evaluation of human mental concentration by using the event-related potential

If subjects performed mental concentration, the latency of P300 event-related potentials was reduced.

Evaluation of human strain and relaxation

The power spectral density in a low-frequency component increased after stress loading.

Evaluation of human stress by using platelet aggregation

We studied the fundamental factors that influence platelet aggregation. We observed an increase in platelet aggregation after the subjects smoked cigarettes.

Publications

Toshima H, Kimura N, Kurihara S. Influence of smoking on the human autonomic nervous system. *Autonomic Neuroscience: Basic Clin* 2009; **149**: 72

Toshima H, Tsukui T (Tokai Univ), Kobayashi Y (Daiichi-Sankyo), Kurihara S. New event-related potential (ERP) induced by loading of auditory and visual stimuli. *J Physiol Sci* 2009; **59 Suppl**:

193.

Ohira T¹, Terada M¹, Kawano F¹, Nakai N¹, Ochiai T (Mitsubishi Heavy Industries), Gytoku J (Tsurui Chemical Co), Sudoh M, Ohira Y¹ (¹Osaka Univ). Region-specific responses of adductor longus muscle to unloading and reloading in wistar hannover rats. *J Physiol Sci* 2009; **59 Suppl**: 216.

Department of Orthopaedic Surgery

Division of Sports Medicine

Keishi Marumo, *Professor*

Hiroki Funasaki, *Assistant Professor*

General Summary

Clinical Research

The ongoing research in our department concentrates on competitive athletes (including professionals), amateurs who include sports activities in their daily lives, and young athletes engaged in school sports clubs or dedicated to training within sports clubs. In 2009 we focused on the issues related to the sports injuries of young athletes.

Research Activities

Three-dimensional motion analysis of the pelvis during soccer kicks

We performed a 3-dimensional motion analysis (200 Hz) of the pelvis during soccer kicks using a Vicon motion capture system (Vicon, Centennial, CO, USA) to evaluate the mechanical stresses around the pelvis. In less than 1 second after the ball impact until extension of the knee, the pelvis provided a “reverse motion,” that is, a rotation towards the anterior tilt in the sagittal plane and a posterior rotation in the axial plane. We concluded that the pelvis plays an important role as the center of the kinematic chain during soccer kicks and acts as a source of additional power providing enough rotation in the sagittal and axial planes. We suspect that the “reverse motion” in the sagittal plane contributes to injuries affecting the hip flexors.

Issues related to sports injuries during growth periods in children and teens

We reviewed major orthopedic sports injuries that occur during various growth periods in school children and teenagers and propose a multifactorial problem-solving approach for their treatment. For the majority of sports injuries, early detection can lead to complete recovery without complications and to an early return to the original activity. Musculoskeletal function in children and teens has recently gained increased attention, and a nationwide school screening survey has been attempted. With more children and teens involved in sports activities, efforts must be made to provide the best long-term support, with good communication among physicians, coaches, and parents.

Publications

Funasaki H, Kan I, Morohashi M, Kato S, Iwama T, Yui N, Marumo K. A case of osteochondral injury of the glenoid rim in a boxing player (in Japanese). *Nippon Seikeigeka sports Igakkai Zasshi* 2009; **29**: 64–8.

Funasaki H, Yoshida M, Kan I, Kato S, Kasama K, Ishizuka R, Marumo K. The effectiveness of

conservative treatment in non-operative patients with a full-thickness tear of rotator cuff (in Japanese). *Katakansetsu* 2009; **33**: 697–700.

Kan I, Funasaki H, Yoshida M, Kato S, Kasama K, Marumo K. Morphological and immunohistological study of the acromion in patients with rotator cuff tear (in Japanese). *Kanto Seikei*

Saigai Geka Gakkai Zasshi 2010; **41**: 11-7.

Books Funasaki H. Sports injuries (in Japanese). In: Abo M, Hashimoto K, editors.

Common and uncommon sense in rehabilitation.
Tokyo: Miwashoten; 2009. p. 55-8.

Health-Care Center

Mikio Zeniya, *Professor and Director*
 Takashi Wada, *Professor*
 Kazumi Kawase, *Assistant Professor*

Yoichi Sakamoto, *Professor*
 Takekazu Onda, *Professor*
 Hiroki Takahashi, *Assistant Professor*

General Summary

Shimbashi Medical Checkup Office

1. “Lung age,” calculated from height, sex, and forced expiratory volume in 1 second, is a new index for expressing respiratory function with age. We assessed the differences in lung age among current smokers, former smokers, and nonsmokers.
2. Healthy habits are represented by Breslow’s 7 healthy practices, Morimoto’s 8 items, and Ikeda’s 6 healthy habits. We assessed which of the 3 classifications of healthy lifestyle habits is the most closely associated with the prevention of metabolic syndrome. Of the 3 models tested, Ikeda’s healthy habits were the most useful for decreasing the risk of metabolic syndrome.

Research Activities

Shimbashi Medical Checkup Office

1. Lung age

We assessed the differences in lung age among current smokers, former smokers, and nonsmokers. The nonsmokers were further divided into 2 groups based on their exposure to second-hand smoke. The lung age minus the calendar age was 9.8 ± 14.7 years in current smokers, 4.8 ± 17.8 years in former smokers, and -0.8 ± 14.0 years in nonsmokers. These data showed significant differences ($p=0.0003$). The smoking index was calculated by multiplying the number of years of smoking by the number of cigarettes smoked per day. The lung age of persons with a smoking index ≥ 600 was 13.2 years and was significantly greater than that (7.2 years) of persons with a smoking index < 600 ($p=0.003$). In nonsmokers, the lung age minus the calendar age was -0.5 years among those exposed to second-hand smoke and -1.0 among those not exposed to second-hand smoke. In conclusion, smoking increases lung age.

2. Basic lifestyle

The predominant cause of metabolic syndrome is an unhealthy lifestyle. Healthy habits are represented by Breslow’s 7 healthy practices, Morimoto’s 8 items, and Ikeda’s 6 healthy habits. The aim of this study was to determine which set of healthy habits was most likely to result in a reduced risk of developing metabolic syndrome. A total of 6,765 men and 2,789 women had medical checkups. They completed a lifestyle questionnaire based on the 3 classifications of healthy habits. The responses were divided into 3 groups (poor, moderate, and favorable) according to each of the healthy habit criteria. The incidence of metabolic syndrome was defined in participants who were newly diagnosed during follow-up. The Kaplan-Meier cumulative 7-year incidence was calculated. In women, the healthy habits of Breslow, Morimoto, and Ikeda showed

significant differences in the incidence between the poor and moderate groups and between the poor and favorable groups. In men, a significant difference was observed among the poor, moderate, and favorable groups for Ikeda's healthy habits. However, no significant difference was observed for Breslow's healthy practices. Morimoto's items showed a significant difference only between the poor and moderate groups. Among the 3 models tested, Ikeda's healthy habits were the most useful for decreasing the risk of metabolic syndrome in Japanese.

Publications

Oikawa T, Kamiya A, Kakinuma S, Zeniya M, Nishinakamura R, Tajiri H, Nakauchi H. Sall4 regulates cell fate decision in fetal hepatic stem/progenitor cells. *Gastroenterology* 2009; **136**: 1000-11.

Wada T, Fukumoto T, Ito K, Hasegawa Y¹, Osaki T¹ (¹Hitachi Ltd). Relationship between the three kinds of healthy habits and the metabolic

syndrome. *Obes Res Clin Prac* 2009; **3**: 123-32.

Wada T, Fukumoto T, Ito K, Hasegawa Y¹, Osaki T¹, Ban H¹ (¹Hitachi Ltd). Of the three classifications of healthy lifestyle habits, Which One is the most closely associated with the prevention of metabolic syndrome in Japanese? *Intern Med* 2009; **48**: 647-55.

Premedical Course

Japanese

Ikuko Noro, Associate Professor

General Summary

Suitability for patients of informed consent documents written in Japanese

A survey was performed to investigate simulated situations: 1) the overall effects of comprehensibility of informed consent documents on patient outcomes; 2) whether or not, and in what ways, affective verbal expressions that moderate patient anxiety and demonstrate partnership or concern are likely to affect patient outcomes; and 3) in what ways patient outcomes may be seen to be correlated. (Publications 1)

Effects of the sex of physicians on medical communication

To assess how the sex of medical students affect the communication styles of medical students and simulated patients, medical interviews, as part of the objective structured clinical examination, performed by 82 (53 male, 29 female) fourth-year medical students at Nagoya University School of Medicine were analyzed with the Roter Method of Interaction Process Analysis System. (Publications 2)

Publications

Noro I, Muramoto T. Correlation of comprehensibility of informed consent documents with and without affective verbal expressions and measured patient outcomes: a survey on students. (in Japanese). *Nippon Hoken Iryo kodo Kagakkai Nenpo* 2009; **24**: 102-16.

Noro I, Abe K, Ban N. Effects of medical students' gender on communication during OSCE medical interviews (in Japanese). *Igaku Kyoiku Jpn* 2010; **41**: 1-6.

Reviews and Books

Ishikawa H, Abe K, Noro I, Takayama T. Functional Approach to Medical Communication Study (in Japanese). In: Fujisaki K, Hashimoto H, editors. Multiple Approaches to Empirical study on Medical Communication. Tokyo: Shinoharashuppanshinsha; 2009. p. 53-82.

Social Science (Law)

Ryuichi Ozawa, Professor

General Summary

Problems of constitutional law in present-day Japan.

Research Activities

I address the problem of constitutional law in present-day Japan, especially pacifism, parliamentary democracy, public finance, right of free speech, and the judicial system. I participate in the “Unequal Society and Safety Net” working party and “Structural Change of Publics” working party of the Legal Committee of the Science Council of Japan.

Publications

Ozawa R. Memories of War as ideal base of Pacifism (in Japanese). In: Urata I, Shimizu M, Miwa T, editors. *Heiwa to Kenpou no Genzai*. Tokyo:

Nishida-Shoten; 2009. p.179–99.

Ozawa R. On Japanese Law School (in Japanese). *Nihon no Kagakusha* 2009; **44**: 4–9.

Human Science

Fukuyama Takao, *Professor*

General Summary

The study of Western philosophy and ethics.

Research Activities

An Acceptance of Others

Contemporary philosophers, for example, Martin Buber, Emmanuel Levinas, Viktor Emil Frankl, and Erich Fromm, contributed new concepts of human relations. They commonly insisted on the passivity of human kind. They criticized the isolation of the self, which Descartes certified, while that self is a cause of narcissism or egoism. Rather, the philosophers offered the self that is embedded in the human relations, and they made anew many rewarding ideas.

Publications

Fukuyama T. The relation between Philosophy and Spirituality: “Acceptance of the Other” and

its’ thinkers (in Japanese). *Igaku Rinri to Tetsugaku* 2009; **6**: 28–31.

English

Osamu Ohara, *Professor*

Tetsuro Fujii, *Associate Professor*

General Summary

English audiovisual education and the history of the English language (Ohara)

English language communication and education: Material analysis and development (Fujii)

Ohara continued his study of graphology and morphology in the letters of the Celys and the Stonors in the fifteenth century. Ohara also continued an investigation of how to make useful digital images and XML files of fifteenth century manuscripts, especially of the *Stonor Letters*. The results of this investigation were discussed in papers read at an international conference.

Fujii analyzed the linguistic similarities between English and Japanese which facilitate learning. He also studied the relationship between reading comprehension skills and testing methods. In addition, he developed self-study materials based on the analysis of question items found in the Test of English for International Communication.

Research Activities

Ohara presented a paper at a session titled “The Rhetoric of Genre” of the International Medieval Congress 2009 held at the University of Leeds in the United Kingdom. In this historical-sociolinguistic study of the *Stonor Letters*, Ohara focused on the usages of auxiliaries and showed how they are different. Ohara visited the National Archive in Britain and studied the *Stonor Letters*. Making use of the results of the study, Ohara continued his research of the graphemes of the letters of the Stonors.

Fujii presented a paper about the lexical and syntactical commonalities between English and Japanese at a conference of the Association for Japanese and English Language and Culture at Meiji University in June 2009. He also presented about the influence of test formats on reading subskills, related to vocabulary, syntax, and inference, at the 35th annual meeting of the Japan Society of English Language Education held at Tottori University in August 2009. In addition, Fujii compiled 3 vocabulary textbooks for second-language learners, based on teaching theories and research findings on vocabulary usage on the Test of English for International Communication.

Reviews and Books

Fujii T. Learn by solving: TOEIC TEST English Words 2260 (in Japanese). Tokyo: Kiriara Publishing; 2009.

Fujii T, Miyano T (Kansai Gaidai Coll). Ultimate vocabulary lite for TOEIC TEST score 500 (in

Japanese). Tokyo: Goken Publishing; 2009.

Fujii T, Miyano T (Kansai Gaidai Coll). Ultimate vocabulary lite for TOEIC TEST score 600 (in Japanese). Tokyo: Goken Publishing; 2009.

German

Yoshiaki Shirasaki, *Professor*

General Summery

1. I have continued educatinal activities for the purpose of verbal and nonverbal communication. These activities are also connected with the aim of the development of moral and philosophical ability of the field of interectural relationships.
2. I have also continued a large translation of Wilhelm Diltthey. This Translation has the following aim, that the so many fiels like ontology, epistemology, metaphysic, religious philosophy and aesthetic etc. at once in one view unify.

Publications

Miyashita K, Shirasaki Y. Die Entstehung des modernen Geisteswissenschaften. Wilhelm Dilt-

ey Saemtliche Werke Bd, 7. Tokyo: The University Press of Hosei; 2009. p.943.

Mathematics

Katsuya Yokoi, *Professor*

Hiroshi Shiraishi, *Assistant Professor*

General Summary

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

Research Activities

1. For $n \geq 1$, given an n -dimensional locally $(n-1)$ -connected compact space X and a finite Borel measure μ without atoms at isolated points, we have proven that for a generic (in the uniform metric) continuous map $f: X \rightarrow X$, the set of points that are chain recurrent under f has μ -measure zero.
2. When financial returns are supposed to be vector-valued non-Gaussian stationary processes, optimal portfolios for general utility function depend on their higher-order cumulants. Assuming that the concerned optimal portfolio depends on the mean, variance, and third-order cumulants of the return process, the asymptotic distribution of a sample version estimator of the optimal portfolio is derived.

Publications

Yokoi K. The size of the chain recurrent set for generic maps on an n -dimensional locally $(n-1)$ -connected compact space. *Colloquium Mathematicum* 2010; **119**: 229–36.

Shiraishi H, Taniguchi M (Waseda Univ). Statistical estimation of optimal portfolios depending on higher order cumulants. *Annales De L'I.S.U. P* 2009; **53**: 3–18.

Physics

Koichi Satoh, *Professor*

Katsumi Kasono, *Assistant Professor*

General Summary

1. Dipalmitoylphosphatidylcholine (DPPC) membranes have been studied from several points of view, including form formation, liquid crystal, phase transitions, interaction with ions, and optical characteristics.
2. Phase transitions, critical phenomena, interacting many-body systems, and computer simulation.

Research Activities

Ripple phase of the DPPC membrane

The ripple phase of the DPPC membrane is assumed to be caused by about 20% fluid lipids in the gel phase. This assumption explains consistently both the surface charge density of multilamellar vesicles and the dielectric increment of unilamellar vesicles during the ripple phase.

Monte Carlo simulations of the $q=10$ Potts model

We have performed cluster update simulations to study systems with the first-order phase transition. We calculated latent heat and magnetization. The results were consistent with the theoretical prediction to 5 digits.

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

Molecular design of gadolinium (III) sugar ball complexes for highly sensitive magnetic resonance imaging agents

Gadolinium (III) heterocyclic complexes are used as the magnetic resonance imaging agents because of the high spin multiplicity to catalyze spin-spin relaxation of excited water protons. Modification of the heterocyclic ligands with gluconic acid moieties improves imaging sensitivity and allows smaller doses of toxic imaging agents. We performed computational analysis of the mechanism of spin relaxation by the Gd (III) complex. Density functional theory calculation revealed that the ligand exchange on Gd^{3+} ion occurs via a concerted process similar to the $\text{S}_{\text{N}}2$ reaction of organic compounds with a low energy barrier.

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 to incorporate and remove 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. The amino acid-Ca carboxylate salts were found to be the most effective of the carboxylate salts of amino acids tested for coupling with Boc-amino acid active esters in organic solvents, such as N,N-dimethylformamide and dimethylsulfoxide.

Review

Okano T. Design and fabrication of functional self-assembled monolayer materials (in

Japanese). *Kagaku Kogyo* 2009; **60**: 441-6.

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

In vivo pollen tube growth of *Chamaecyparis pisifera*.

1. Nucellar cell death in response to pollen tube elongation

In *C. pisifera*, the pollen tube grew intermittently for approximately 2 months through nucellar tissue toward the female gametophyte. After germination, the pollen tube grew

for 1 to 3 weeks and formed multiple branches. Growth then ceased for approximately 1.5 months, and only a single branch of multiple branches began to grow again, showing rapid elongation. The single branch grew approximately 300 μm and conveys sperm cells toward the female gametophyte. In the region up to about 100 μm from the nucellar apex, vacuoles in the nucellar cells displayed high internal electron density and contained various vesicles and organelles. The nucellar cells adjacent to the pollen tube underwent cell death with DNA fragmentation. These results suggested that autophagy occurred in the region up to about 100 μm from the nucellar apex and that substances accumulated in the vacuoles were released into the extracellular matrix (ECM) with nucellar cell death. The substances might be reused as a source of nutrients during pollen tube growth.

2. Distribution of molecules involved in angiospermous pollen tube elongation in the nucellus

Arabinogalactan proteins (AGPs) and pectins in the styles of angiosperms support pollen tube growth. In this study, distributions of pectin, AGPs and pectinases in the pollen tube and nucellus of *C. pisifera* were investigated using an antibody (JIM5) that recognizes unesterified pectins, an antibody (JIM7) that recognizes methyl-esterified pectins, an antibody (JIM13) that recognizes AGPs, and antibodies (Cry j 1 and Cry j 2) that recognize pectinases. Unesterified pectins and methyl-esterified pectins were distributed in cell walls and the ECM throughout the nucellus. Cry j 1 was widely distributed throughout the whole nucellus, whereas pollen tube walls were devoid of signal. Cry j 2 was located mainly in the pollen tube walls. These findings suggest that Cry j 1 is secreted mainly by nucellar cells, that Cry j 2 is secreted by pollen tubes, and that both enzymes facilitate the growth of the pollen tubes by degrading the pectins of the cell wall and the ECM. AGPs, which, in tobacco, are secreted in the ECM from transmitting tissue and are thought to form a nutrient source for pollen tube, were distributed in cell walls and the ECM in the region up to about 100 μm from the nucellar apex. We presumed that AGPs in the nucellus of *C. pisifera* play a similar role as they do in tobacco.

School of Nursing

Basic Nursing

Sawako Haga, *Professor*
Mayumi Kikuchi, *Assistant Professor*

Machiko Hirao, *Associate Professor*
Chieko Hanyu, *Assistant Professor*

Research Activities

The research activities of the basic nursing group can be divided into the following 3 areas.

Haga has been investigating the effects of physical assessment by nurses, the evidence of nursing skill, and the history of nursing.

Hirao has been investigating the effects of nursing and Florence Nightingale's thoughts about nursing.

Kikuchi has been investigating the effects of nursing education, methods of teaching, and nursing diagnosis.

Hanyu has been investigating the effects of physical assessment by nurses, the evidence of nursing skill, and stoma management.

Review and Books

Kikuchi M, Hanyu C, Haga S, Hirao M, Aoki N. Analysis of physical assessment in clinical training : focusing on the assessment contents of nursing students. (in Japanese). *Nippon Kangogaku Kyoiku Gakkaishi* 2009; **19**: 111.

Hanyu C, Kikuchi M, Haga S, Hirao M, Aoki N. Analysis of physical assessment in clinical training : focusing on the motivation of nursing students. (in Japanese). *Nippon Kangogaku Kyoiku Gakkaishi* 2009; **19**: 111.

Haga S, Hirao M, Ebina F. A Study of Dr. Kanehiro Takaki's thought on health education (4): from his opinions related to the improve-

ment of girls' education in "Rinji Kyoiku Kaigi" (in Japanese). *Nippon Ishigaku Zasshi* 2009; **55**: 204.

Ebina F, Haga S, Hirao M. The early nursing system in Tokyo Charity Hospital (in Japanese). Proceedings of the 22th Academic Conference Japan Academy of Nursing History 2009.

Nakaki T, Kuroda Y, Kuroda Y, Honjo K, Nakano Y, Tanahashi Y, Matsumoto C, Uchida M, Yamada M, Hayashi M, Fujisaki K, Kikuchi M. Intelligible Middle-range theory (in Japanese). Tokyo: Gakken; 2009.

Nursing Administration

Sugino Oishi, *Professor*

General Summary

To clarify the characteristics of the Japanese nursing system after World War II, I have been studying the history of nursing and analyzing the factors affecting the Japanese nursing system.

Research Activities

Quantitative analysis of changes in the numbers of nursing graduates and assistant-nursing graduates in Japan from 1974 to 2006

The purposes of this study were: 1) to analyze changes in the numbers of nursing graduates (from nursing universities, nursing colleges, and nursing schools), the numbers of assistant-nursing graduates (from assistant-nursing schools and nursing high schools), the ratios of nursing graduates to nursing graduates and assistant-nursing graduates (nursing graduation ratio); and 2) factors that caused the changes from 1974 through 2006 in Japan. For this analysis, official data were used. Prefectures were classified into 4 groups on the basis of: 1) the ratio of nurses to nurses and assistant nurses (nursing ratio) and the nursing graduation ratio, 2) the nursing ratio and the number of nursing graduates, and 3) the nursing ratio and the number of assistant nursing graduates. The results were as follows. 1) From 1974 through 2006, the number of nursing graduates per 100,000 persons and the nursing graduation ratio increased in all prefectures. 2) The nursing graduation ratio depended on the medical and nursing structures in the prefectures. 3) In many prefectures with a high nursing ratio, the number of nursing graduates was low. This association was thought to be due to the transfer of nursing graduates.

Study of the policy of the Nursing Affairs Division GHQ by analyzing censorship by General Headquarters Supreme Commander for the Allied Powers

Records of the censorship of medical and nursing publications from 1945 through 1949 in occupied Japan are thought to be housed in the Gordon W. Prange Collection at the University of Maryland. The purpose of this study was to investigate the circumstances of publishing and the criteria for censorship by General Headquarters Supreme Commander for the Allied Powers (GHQ) in occupied Japan. I visited the Gordon W. Prange Collection and collected and analyzed its newspapers in 2009.

Historical research on Japanese nursing reform after World War II

The Nursing Affairs Division of GHQ revised the nursing law and the nursing system during the occupation. The present Japanese nursing system is based on the nursing reforms of the GHQ but has been modified because of economic and educational problems. I obtained information from the GHQ records and related persons to investigate nursing reforms.

I introduced the methods of historical investigation and presented the results of my study to students in my lecture on nursing management.

Publications

Oishi S. Quantitative Analysis of the changes in the numbers of nursing graduates and assistant-nursing graduates in Japan from 1974 to 2006 (in Japanese). *Tokyo Jikeikai Ikadaigaku Zasshi* (Tokyo Jikeikai Med J) 2009; **124**: 201-12.

Oishi S. Analysis of the Gordon W. Prange collection pamphlet list concerning medicine and nursing (in Japanese). *Nippon Ishigaku Zasshi* 2009; **55**: 202.

Adult Nursing

Shoko Fujino, *Professor*
Naomi Takashima, *Professor*

Ryuko Fujimura, *Professor*
Chie Watanabe, *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 adopt these results and reflect 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 I call “caring touch,” is used to strengthen the relationship between nurses and patients and to ease suffering.

Takashima clarified the present status of perioperative nursing for gastrointestinal surgery with the shortening of hospital stays. Also, we analyzed patients’ quality of life related to gastrointestinal surgery. The present study gathered concrete data that can be used to develop high-quality perioperative nursing that can be implemented in a short period of time in clinical settings.

Watanabe has performed a national survey on nursing care for lymphedema after cancer therapy. Also, we have performed an interventional study to evaluate the practicality and validity of standard education programs on nursing care for lymphedema.

Publications

Takashima N, Gokita K. The present status of perioperative nursing in the digestive organ surgery under hospital-days shortening-Recognition of the head nurses by national investigation (in Japanese). *Nippon Critical Care Kango Gakkaishi* 2009; **5**: 60–8.

Takashima N, Gokita K, Hamada A, Yamada M, Watabe S, et al. The symptom management and patient education of day surgery patients (in Japanese). *Yokohama Kangogaku Zasshi* 2009; **2**: 33–40.

Gokita K, Takashima N, Watabe S, Kanno H, et al. A study of on assessment of healthy-related QOL and its related factors in families if brain tumor patients (in Japanese). *Yokohama Kangogaku*

Zasshi 2009; **2**: 17–24.

Nagata M, Takashima N, Oga A, Tanaka N, et al. The cultivation of subjectivity about clinical nursing education by participation experience-based practical instructor workshop (in Japanese). *Yokohama Kangogaku Zasshi* 2009; **2**: 41–7.

Watabe S, Takeda N, Takashima N, Gokita K, Oshige K. The relationship between surgical infection and skin preparation methods before total hip arthroplasty in Japan (in Japanese). *Nippon Seikeigeka Kango Kenkyukaishi* 2009; **4**: 68–75.

Terui Y¹, Mishima Y¹, Sugimura N¹, Kojima K¹, Sakurai T¹, Mishima Y¹, Kuniyoshi R¹, Taniyama A¹, Yokoyama M¹, Sakajiri S¹, Takeuchi K¹,

Watanabe C, Takahashi S¹, Ito Y¹, Hatake K¹ (¹*Cancer Inst Hosp*). Identification of CD20 C-terminal deletion mutations associated with loss of CD20 expression in non-Hodgkin's lymphoma. *Clin Cancer Res* 2009; **15**: 523-30.

Reviews and Books

Watanabe C. Nursing and Sexuality: hematological cancer and sexuality (in Japanese). *Nippon Seikagakkai Zasshi* 2009; **27**: 75-7.

Gerontological Nursing

Miyoko Sakurai, *Professor*

Kumiko Date, *Associate Professor*

General Summary

The main research activities in our department have focused on the psychology of families of elderly persons with dementia and the relationship between health and lifestyle in elderly adults.

Research Activities

Psychological changes in families of elderly persons with dementia who are entering institutions

Semistructured interviews were conducted of 10 family caregivers residing in urban and rural settings. Data were qualitatively analyzed using a grounded theory approach. The results of the analysis were as follows. While families were suffering with the social norm of caring for parents, they became aware of the intimate relationship between parents and children. This experience changed the feelings of the family caregivers from a sense of guilt to a sense of gratitude for their parents. Results of this study were presented at the 14th Annual Scientific Meeting of the Japan Academy of Gerontological Nursing and the Japanese Society for Dementia Care in 2009.

Date has been investigating health status and various factors influencing it in middle-aged and elderly adults for primary and secondary prevention of lifestyle-related diseases from a comprehensive perspective, including nutrition, exercises, and rest.

Reviews and Books

Date K. Management and education of the enteral and parenteral nutrition (in Japanese). In: Nakamura M, Hasegawa K, editors. Nutri-

tion sciences for clinical and home care. 3rd ed. Tokyo: Nouvelle-Hirokawa; 2009. p. 249-74.

Mental Health and Psychiatric Nursing

Masashi Kawano, *Professor*

Setsuko Hayashi, *Assistant Professor*

Research Activities

Education and research

In education, we continued reconstruction to collaborate lecture, laboratories, and practicums for a second year. For freshmen, we gave lectures about the concepts of mental health and current issues. For sophomores, we gave lectures work with psychiatrists classes, and focused on the diagnosis and nursing care of psychiatric patients. For juniors, education activities included mainly laboratories for communication skills, interpersonal relationships, and the nursing process using a DVD produced by our department and Kichijoji Hospital utilize for following practicum. For the senior-year comprehensive practicum, we redefined the goals and objectives for deeper understanding of and the use of skills for psychiatric patients.

In research, we completed a study entitled "Child and Adolescent psychiatric and Mental Health Networking in Kanagawa" and presented the findings at the second conference of Japan Community Collaboration in Psychiatric Mental Health Nursing in Tokyo, held at the The Jikei University. Others studies that continued were entitled "Comparison between Japan and Thailand in Psychiatric Mental Health Care for Rural Patients," and "Child and Adolescent Psychiatric Care in Itabashi-ku, Toshima-ku, and Nerima-ku, Tokyo," and "Communication Skills Needed for Undergraduate and Graduate School in Nursing."

Evaluation

For a second year, our department has redefined the contents of education. We believed the changes were effective. We will require 1 more year to reform the entire contents. A DVD we produced contributed to learning in the classroom and in practicums and strengthened the relation between the clinical hospital and the nursing school. It is important to continue to apply evaluations from students to improve lectures, laboratories, and practicums. Next year we plan to develop stronger ties with laboratories and practicums and to develop educational materials for the future.

Research activities must remain oriented toward psychiatric mental health. It is essential to apply research results to education.

Publications

Ichinoyama R, Murakami M, Funasaki K, Makino K, Myojin K, Ueno E, Kawano M. Psycho-Education approach towards hallucinated patient to support self coping behavior (in Japanese). *Kyosotukushi* 2009; **4**: 1-10.

Funasaki K, Ichinoyama R, Kawano M, Takano E, Kamihira E, Ueno E. Psychiatric clinical report

Experimental learning about patient's well-being is not only nurses words-caring based from own relaxing experience utilize to care for others (in Japanese). *Rinsho Kango* 2009; **35**: 811-5.

Iwasa T, Imai M, Shimizu Y, Kawano M. Psychiatric clinical report. The possibility to nursing utilize animal therapy; Communication and be-

havioral change uncommunicable schizophrenia patients (in Japanese). *Rinsho Kango* 2009; **35**: 956-62.

Tanaka A, Kawano M. To search caring element in ICU-To Find out caring from patients and nurses concepts of care and heart (in Japanese). *Rinsho Kango* 2009; **35**: 1097-8.

Sen H, Ichinoyama R, Kawano M. Psychiatric clinical report To assist a schizophrenia patient to out burst own feelings which could not open to anybody; To prepare space to speak out (in Japanese). *Rinsho Kango* 2009; **35**: 1394-9.

Moriizumi Y, Kabayashi K, Kawano M. Psychiatric clinical report The reality for men who take care of their wife with demetia; support husbands by their tail (in Japanese). *Rinsho Kango* 2009; **35**: 1689-94.

Miyahara N, Kobayashi K, Haruhara A, Kawano M. Psychiatric clinical report Relaxing music, auditory stimulation by family voice; Care for patients who are unexpressed emotions (in Japanese). *Rinsho Kango* 2009; **35**: 2078-83.

Ichinoyama R, Goto M, Funahasaki K, Kawano M, Murata M, Yoshida M, Myojin K, Ueno E. Psychiatric clinical report Outcome to utilize foot care and change of self care level (in Japanese). *Rinsho Kango* 2009; **35**: 2215-24.

Kyotani K, Ichinoyama R, Funasaki K, Kawano M. Psychiatric clinical report Serving strait coffee for a terminal stage cancer patient, improvement the interpersonal relationships (in Japanese). *Rinsho Kango* 2010; **36**: 251-7.

Kakegawa Y, Sasaki K, Sato H, Kawano M. Psychiatric clinical report Intent and meaning of psychiatric home visiting, analysis of question-

naire by client (in Japanese). *Rinsho Kango* 2010; **36**: 370-3.

Kawano M. Clinical practice in child and adolescent psychiatric and mental health nursing-now what else needed in child and adolescent psychiatric mental health nursing (in Japanese). *Shoni Kango* 2009; **32**: 1155-6.

Kawano M. Clinical practice in child and adolescent psychiatric and mental health nursing-nursing and community support, to care for supporting family members (in Japanese). *Shoni Kango* 2009; **32**: 1193-7.

Reviews and Books

Kawano M, editor. Psychiatric clinical nursing skill (in Japanese). Tokyo: Chuo Hoki Shuppan; 2009.

Yoshimatsu K, Koizumi N, Kawano M, editors. Psychiatric Mental Health Nursing I Mental Health Nursing (in Japanese). 5th ed. Tokyo: Nuebel Hirokawa; 2010.

Kawano M, editor. Psychiatric Mental Health Nursing II Psychiatric Nursing (in Japanese). 5th ed. Tokyo: Nuebel Hirokawa; 2010.

Kawano M. Jean Watson (in Japanese). In: Kobayashi F, Higuchi Y, Kodama K, et al, editors. Inquires in modern nursing, Person and philosophy. Tokyo: Japanese nursing association publisher; 2009. p. 251-81.

Kawano M, editor. Study of community collaboration mental health nursing. England-U.S.A.-Japan three countries community mental health nursing (in Japanese). Tokyo: Seron Jiho; 2009.

Child Nursing

Kiyo Hamanaka, *Professor*

Kayo Cho, *Associate Professor*

Research Activities

Development and verification of an educational program to support continued work in clinical child care

Hamanaka held a training seminar in Aomori-shi based on a tentative plan to help nursing students continue working after graduation. Hamanaka's follow-up of participants found that a training seminar was effective against "reality shock."

A study of the organization of nursing practice for outpatients and of outpatient nursing to promote the health of children in basic education

As a co-author of a study with the support of a Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Hamanaka presented results of a study from last year at a congress and chaired a workshop at the annual

meeting of the Society of Ambulatory and General Pediatrics of Japan. Hamanaka has started to prepare a report.

A study to build a support system for families of children with intractable illnesses

Hamanaka analyzed data from the interviews of families of children with intractable illnesses, and considered ways of supporting these families.

The social growth process of children with end-stage renal disease and drafting a support plan by offering information

Cho interviewed children with end-stage renal disease and their parents to clarify methods to deal with problems that patients and parents face in the process of social growth. Cho presented her results at the 31th congress of the Japanese Society for Pediatric Renal Failure.

Publications

Cho K. Mother's perception and support about the school life of pediatric post renal transplant

patient (in Japanese). *Nippon Shouni Jinfuzen Gakkaizasshi* 2009; **29**: 249-51.

Maternity Nursing

Kimiko Kayashima, *Professor*

Yasuko Hososaka, *Assistant Professor*

General Summary

Studies have been performed to examine the various health issues in each stage of a woman's life and to explore how nursing assistance should be extended in maternal nursing.

Research Activities

The relationships between menstrual symptoms and mind-body characteristics in adolescent girls

This study of 323 adolescent girls analyzed the relationships among physique, lifestyle, health attitudes, gender role perception, and other factors to clarify mind-body factors related to menstrual symptoms. Approximately 80% of the target group was of average build, but half perceived themselves to be overweight. An analysis of the relationship with menstrual symptoms (Menstrual Distress Questionnaire Scale) revealed significant correlations with body-mass index ($p < 0.05$), family factors ($p < 0.05$), and chance factors ($p < 0.05$) related to the subjective sense of health control (Health Locus of Control Scale) in premenstrual high school students, and a significant correlation with chance factors on the Health Locus of Control Scale ($p < 0.05$) for those in the middle of the menstrual cycle. Among university students, there was a significant correlation with

negative attitudes toward menstruation ($p < 0.01$), self-care ($p < 0.05$), and androgyny ($p < 0.01$) as pertains to gender role personality (Bem Sex Role Inventory Scale) in both those who were premenstrual and those in the middle of the menstrual cycle.

Current situation and trends in research related to sexuality in nursing

The research literature related to sexuality in nursing published from 1985 through July 2009 was reviewed. Research and interpretations concerning nurses' understanding of the subject and the understanding of nurses and nursing students of sexual dysfunctions of various types of patients (users) are available, but the majority of reports are case studies involving small numbers of patients, and there has been little research related to evaluation of care.

Quality evaluation of midwifery care

Using the "Quality evaluation of midwifery care in medical institutions: Evaluation criteria for self-evaluation — 2nd edition," a survey was performed of the quality of midwifery care among midwives belonging to the Japanese Nursing Association. The results showed that levels of self-evaluation in midwifery care increased with the length of experience and the number of deliveries attended but that self-evaluation on care during pregnancy was low overall and that self-evaluation levels tended to be low on mixed wards and among graduates of nursing universities.

Investigation of the degree of satisfaction of university instructors and related factors

We are preparing a study of the degree of satisfaction among university instructors engaged in practical training for maternity nursing and relevant factors. The study will focus on maternity nursing instructors at 4-year universities in Japan. We are also preparing to employ a questionnaire on nursing university teachers' self-efficacy along with a simple questionnaire on occupational stress.

Publications

Nukita H, Taniguchi C, Emisu F. Relationship between individual attributes of midwives in perinatal care and the use of gloves (in

Japanese). *Nippon Josan Gakkaishi* 2009; **23**: 208-16.

Community Health Nursing

Noriko Okuyama, *Professor*

Juniko Shimazawa, *Associate Professor*

General Summary

The major research projects in our department have been focused on: 1) the learning achievements of public health nurses and 2) elucidating methods of public health nursing care for mentally ill patients living at home.

Research Activities

Public health nursing education

This study aimed to develop an essential skills framework and to determine the achievement levels necessary for students graduating from schools that provide basic education for obtaining a license as a public health nurse in Japan.

Public health nursing care for mentally ill patients living at home

This study aimed to elucidate methods of public health nursing care for mentally ill patients living at home. Data were gathered through semistructured interviews of 5 public health nurses belonging to the handicapped person's welfare division. The analysis is in progress.

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