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Introduction

It is my great pleasure to publish *Research Activities 2010*, which is a report on the scientific and educational activities at The Jikei University School of Medicine in 2010. 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 2010. In this issue, only selected papers published by each department, institute, and laboratory are listed at the end of each report owing to limitations of space. Similarly, the names of staff are limited to assistant professor and above.

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

Sei-I-Kwai was a medical group organized in January 1881 by persons interested in promoting medical studies. Kanehiro Takaki, the founder of The Jikei University School of Medicine, was elected its president. In May 1881, Sei-I-Kwai established a medical school, which was the beginning of The Jikei University School of Medicine. Kanehiro Takaki pointed out the importance of nutrition to prevent beriberi, which was a serious disease in Meiji era. He performed the first clinical trial in Japan to prove that the quality of food is a key factor to prevent beriberi. Thus, our university has a long tradition of clinical study and has been encouraging its staff to perform research, including clinical study and basic science.

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

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

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

Satoshi Kurihara President The Jikei University School of Medicine

November 5, 2011

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

Activities

- Registered members: 232 (as of April 1, 2011) Members using the Center: 140/year Telephone service: 88 cases
- The 31st summer seminar was held on August 7, 2010. Seventy-six persons participated.
- 3. Monthly seminars were held on the second Saturday afternoons of the month in April, May, June, July, September, November, February, and March. Twenty-five to 30 persons attended each seminar.
- 4. The "CME Center News" is mailed monthly to the registered members.

Center for Medical Education

Osamu Fukushima, Professor and Director Mariko Istubo, Professor Kazunori Utsunomiya, Professor Tetsuya Kawamura, Associate Professor Masato Matsushima, Associate Professor Machiko Hirao, Associate Professor Hiroyuki Takahashi, Associate Professor Yoshio Ishibashi, Assistant Professor Naofumi Kimura, Professor Hideaki Kashiwagi, Professor Sugino Oishi, Professor Hisashi Onoue, Associate Professor Mariko Nakamura, Associate Professor Nobuyuki Furutani, Associate Professor Toshikazu Sakuyama, Assistant Professor

General Summary

The Office of Educational Development was founded in 1999. Staff members were recruited from the School of Medicine. Its main interests were analysis of medical education reports published by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), the Ministry of Health, Labour and Welfare (MHLW), and medical associations; technical support of faculty, management of faculty development and education seminars; and the implementation of tutorials, objective structured clinical examinations (OSCEs), and community-based medical education programs in 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 health-care workers. In 2005, the Office of Educational Development was reorganized as the Center for Medical Education. The Center consists of the Office of Medical Education, the Office of Nursing Education, the Office of Postgraduate Clinical Training, and the Office of Educational Development. Furthermore, the secretariat was set up in the Center in April 2006. The Office of Medical Education contributed to revisions of the undergraduate curriculum, to implementation of OSCEs in years 4 and 5, and to faculty development programs (writing multiple-choice questions, educational techniques at clinical setting, and rater training for OSCEs). The Office of Nursing Education contributes to faculty development programs for nursing teachers (physical assessment training). The Office of Postgraduate Clinical Training supports the management of residency programs and implementation of faculty development programs for attending physicians belonging to 4 attached hospitals. The Office of Educational Development helped establish an e-Learning system for students and health-care providers in the community, manages 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. Our proposal, "Learning assessment system for advancing students' growth," was selected to receive a Supporting Grant for Implementing University Education and Supporting Student Learning Program 2010 by MEXT. This project aims to establish computer-based testing for cumulative tests in the Schools of Medicine and Nursing, and a

portfolio evaluation system to ensure that each student is fit to practice as a healthcare provider upon graduation. The first trial of computer-based testing in February failed because of problems in saver programs and circumstances of the local area network. This project will continue for 3 years. The computer-based testing system will save the costs of printing test papers, and the portfolio system will nurture each student's fitness to practice.

2. We promoted "Develop 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 this 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 clinicpathological conference materials supplied via an intranet system to our medical students and postgraduate trainees. The project ended in 2010. We invited Professor Peter McCrorie of St. George's, University of London, to serve as an external rater in November. We published a final report.

3. We promoted "Research on Improvement of the National Examination for Medical Practitioners" with a Supporting Grant for Developing Community-based Medical Practice 2010 from the MHLW. We examined final OSCEs given at graduation in medical schools in the United Kingdom and offered our data to the MHLW.

4. We promoted "Research on Nurse Practitioners Working with Cancer Specialists (physicians and pharmacists)" with a Supporting Grant for Clinical Cancer Research 2010 from the MHLW. We investigated the roles of nurse practitioners in the United States and Taiwan and sent questionnaires to Japanese nurses about the necessity of nurse practitioners in Japan.

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

6. Contribution to other institutions of higher education (faculty-development lectures and workshops): Waseda University (May), Association of Healthcare Management (June), IMS Patient-Safety Seminar (July), Showa University Education Workshop (July), Tokyo Metropolitan University (August), Kyoto Red-Cross Hospital (August), Association of Occupational Therapy/Physical Therapy Schools in Kyushu (September), Nara Medical School (September), Teacher Training of Judo Therapy Schools held by the MHLW (September), Tokyo Medical University (September), Otaru Ekisaikai Hospital (October), Layering Seminar (October), Iwate Medical University (November), Kokura Rehabilitation School (November), Teacher Training of Occupational Therapy/Physical Therapy Schools held by the MHLW (January), Nara Medical School (February), and Daitobunka University (February).

Publications

Kobayashi-Nagata S, Koyama H, Asai A, Noguchi Y, Maeno T, Fukushima O, Yamamoto W, Koizumi S, Shimbo T. Experiences of alcohol-harassment among medical students. *Med Educ* 2010; **44:** 1213-23.

Reviews and Books

Sakai K, Sawai T, Takizawa T, Fukushima O, Shimada K. Historical development of the system of medical education and medical licensure and its effect on the evolution of medical schools in Japan (in Japanese). *Igaku Kyoiku* 2010; **41:** 337-46.

Fukushima O. Editorial board (in Japanese). In: Nihon Igaku Kyoiku Gakkai, editor. White paper on medical education on 2010. Tokyo: Shinohara Syuppan Shinsya; 2010. p. 271. **Fukushima O.** Evaluation method of problembased learning tutorial (in Japanese). In: Nihon Yakugakkai, editor. Guidebook on Problembased learning in pharmaceutical science students. Tokyo: Kagaku Doujin; 2011. p. 89-96.

Department of Anatomy (Gross Anatomy and Neuroanatomy)

Yoshinori Kawai, Professor

General Summary

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

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 analyses of excitatory and inhibitory synaptic input patterns onto specific cell types were performed with patch-clamp recordings and immunoelectron microscopy. The cell-size distribution and numerical density of cNTS neurons were examined in subregions at levels of the area postrema. In the subpostremal and dorsomedial subnuclei, characterized by the presence of dense glutamatergic and sparse GABAergic somata, small calbindin neurons constituted 42% of all cells. The medial subnucleus contained large numbers of glutamatergic, GABAergic, and catecholaminergic somata, and large tyrosine hydroxylase-containing cells constituted 13% of cells in this region. Small neurons ($<150 \mu m^2$) represented about 80% of the total cell population in the cNTS. Predominant excitatory postsynaptic currents were observed in the small neurons of adults, 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 GAB-Aergic synapses were more frequently associated with dendrites of large catecholaminergic cells (73%) than with those of small calbindin-containing cells (10%). These results indicate that differential synaptic input patterns are developmentally established in distinct small and large neurons.

Local axonal arborization patterns of distinct neuronal types in the cNTS

Neurons in the cNTS are heterogeneous in size (somal area of 50 to 450 μ m²) 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 recordings. According to the patterns of axonal branching behaviors, cNTS cells can be classified into 2 groups: smaller cells (mean somal area, 94.1 μ m²; range, 62–120 μ m²; n=22) and larger cells (mean somal area, 245 μ m²; range, 142–411 μ m²; 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). Larger neurons had more extensive dendritic arborization, expressed by total dendritic length (P<0.01) and 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 local circuits and that large neurons convey the integrated information to other autonomic brain regions.

Postnatal development of GABAergic axon terminals in the rat NTS

The proper function of the brain depends on a precise arrangement of excitatory and inhibitory synapses. Although the cNTS plays a pivotal role in cardiorespiratory reflexes, we know little about the formation of the local neural network in the cNTS. In the present study, we focused on GABAergic axon terminals and investigated postnatal changes in GABAergic synaptic organizations in the rat cNTS immunocytochemically at both the light and electron microscopic levels. The counting of synaptic and nonsynaptic GABAergic axon terminals revealed that the number of GABAergic axon terminals in the cNTS was constant until the second postnatal week and that GABAergic axon terminals were reorganized on about postnatal day 10 (P10). Electron microscopic observation revealed that most GABAergic axon terminals formed axosomatic synapses on neurons with smaller soma (smaller neurons) on P2 to P4 but that the number of axosomatic synapse decreased considerably after P8. Orphan GABAergic boutons were present specifically near somata of smaller neurons on 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 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 prerequisites 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. *N*-methyl-D-aspartate (NMDA) receptor activation is critically implicated in this process, and its blockade leads to disruption of normal circuit formation. This phenomenon has been extensively investigated in several neural systems, including the somatosensory system, but has not yet been evidenced in the visceral sensory system. Ultrastructural analysis of GABAergic synapses and electrophysiological analysis of inhibitory and excitatory postsynaptic currents of cNTS cells revealed that developmental changes in synaptic organization are blocked by MK-801, an NMDA receptor antagonist, when administered on P5 to P8, a presumed critical period for the visceral sensory system. Normal synapse reorganization during postnatal development dictates undifferentiated neonatal cNTS neurons in terms of synaptic input patterns measured with electron microscopy and electrophysiology into 2 cell groups: small cells under far stronger excitatory influence and large cells under far stronger inhibitory influence. Blockade by MK-801 during the critical period might leave adult neurons wired in undifferentiated synaptic networks, possibly preventing synapse elimination and subsequent stabilization of the proper wiring.

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

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

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

GABAergic neurons are major inhibitory interneurons that are widely distributed throughout the central nervous system. The cNTS, which plays a key role in respiratory, cardiovascular, and gastrointestinal functions, 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 2 (VGLUT2), calbindin, and tyrosine hydroxylase (TH) messenger RNAs, the cNTS was divided into 4 subnuclei: the subpostrema and the dorsomedial, commissural, and medial subnuclei. The numerical density and size of somata in the 4 subnuclei were then quantified with an unbiased dissector analysis. Calbindin-positive cells constituted subpopulations of small non-GABAergic neurons preferentially localized in the medial subnucleus. GABAergic neurons constituted a subpopulation of small neurons, preferentially localized in the commissural and medial subnuclei, which represented at least 50% of small cells in these subnuclei. Thus, the GABAergic small neurons were located around TH-positive large cells in the ventrolateral portion of the cNTS. This finding, in combination with the results of previous studies in the rat cNTS showing that large cells originate efferents from the cNTS, suggests that GABAergic small neurons in the commissural and medial subnuclei regulate output from the cNTS.

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

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

Publications

Negishi Y, Kawai Y. Geometric and functional relationships in visceral sensory nucleus. *J Physiol Sci* 2011; **61 Suppl:** S278.

Negishi Y, Kawai Y. Geometric and functional architecture of visceral sensory microcircuitry. *Neurosci Res* 2010; **68 Suppl 1:** e392.

Department of Anatomy (Histology and Embryology)

Masataka Okabe, Professor Toshiaki Tachibana, Assistant Professor Yasuyo Shigetani, Assistant Professor Hisashi Hashimoto, Professor Hideaki Suzuki, Assistant Professor

General Summary

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

Research Activities

Acquisition of glial cells missing 2 enhancers contributes to a diversity of ionocytes in zebrafish

Glial cells missing 2 (gcm2) encoding a GCM-motif transcription factor is expressed in the parathyroid gland in amniotes. In contrast, gcm2 is expressed in pharyngeal pouches (a homologous site of the parathyroid), gills, and H⁺-ATPase-rich cells (HRCs), a subset of ionocytes on the skin surface of the teleost zebrafish. Ionocytes are specialized cells that are involved in osmotic homeostasis in aquatic vertebrates. Here, we show that gcm2 is essential for the development of HRCs and Na⁺-Cl⁻ cotransporter-rich cells (NCCCs), another subset of ionocytes in zebrafish. We also identified gcm2 enhancer regions that control gcm2 expression in the ionocytes of zebrafish. Comparisons of the gcm2 locus with its neighboring regions revealed no conserved elements between zebrafish and tetrapods. Furthermore, we observed gcm2 expression patterns in embryos of the teleost fishes medaka (Oryzias latipes) and fugu (Fugu niphobles), the extant primitive ray-finned fishes polypterus (Polypterus senegalus) and sturgeon (a hybrid of Huso huso×Acipenser ruhenus), and the amphibian African clawed frog (Xenopus laevis). Although gcm2-expressing cells were observed on the skin surface of medaka and fugu, they were not found in polypterus, sturgeon, or the African clawed frog. Our results suggest that the acquisition of enhancers for the expression of gcm2 contributes to the diversity of ionocytes in zebrafish during evolution.

Novel ataxia mouse shows extensive neuropathological changes in the dorsal root ganglion neurons

We have investigated the neuropathological changes in the peripheral nervous system of our novel ataxia mouse, which has a severe gait disorder of the hind legs. On transverse sections of the lumbar spinal nerves, many vacuoles were found in nerve fibers. Longitudinal sections showed swellings and vacuoles in both the spinal nerves and the nerve roots. In the lumbar dorsal root ganglion, the perikarya of some of the larger neurons showed autolysis, whereas those of the smaller neuron appeared to be intact. The shape of the lysed neurons was preserved, and some satellite cells encircled the neuron. No inflammatory cell infiltration was observed. The autolysis of the larger neuron was also found in the cervical and thoracic dorsal root ganglia. These results suggest that the gait disorder of the hind legs of our novel ataxia mouse is caused by a neuropathy of proprioception.

A novel method for analyzing tissue-specific epigenetic memory

This year we attempted to develop recombination-induced tag exchange (RITE), a genetic method that induces a permanent epitope-tag switch in the C-terminal coding sequence of H3.3, a histone H3 variant working as an active epigenetic memory, after a tissue-specific induced activation of causes recombination (Cre) recombinase.

We constructed a vector that ubiquitously expresses hemagglutinin (HA)-tagged H3.3, under a cytomegalovirus promoter without Cre recombinase. The existing Cre recombinase HA-tag at H3.3 C-terminal was exchanged for a 3xFlag-tag.

First, this vector was applied to 293 cells with or without a Cre-expression vector. As expected, HA-tagged H3.3 was incorporated in chromatin without Cre recombinase, whereas Flag-tagged H3.3 was incorporated in chromatin with Cre recombinase.

Next, we constructed a RITE vector working under a muscle-specific creatine kinase promoter by recombination with a bacterial artificial chromosome. We applied this muscle-specific expression vector to the C2C12 cell line. After 7 days of differentiation following transfection, HA-tagged H3.3 was incorporated in chromatin without Cre recombinase, whereas Flag-tagged H3.3 was incorporated in chromatin with Cre recombinase.

Development of a new culture method for a precursor to the neural crest and pre-placodal ectoderm

The vertebrate-specific structures neural crest and sensory placodes arise from a region of the embryonic ectoderm that lies between the neural plate and the future epidermis. We developed a new culture method for a precursor to both the neural crest and the pre-placodal ectoderm; the cultured cells characteristically express Dlx5, which is a neural plate border specifier that non-cell-autonomously positions the neural crest shown by Slug/ SOX2/3 and the future epidermis shown by keratin19/GATA3. The original method for producing the neural crest cells is to add bone morphogenetic protein (BMP) 4 into the medium of grafts, which are dissected from the neural plates of stage 6 to 7 chicken embryos. An additional recombinant protein, fibroblast growth factor (FGF) 2, leads to increased expression of neural plate border-specific molecular markers Dlx5, and moderately increased expression of Six1 and Eya2, following morphological change to the simple squamous epithelium. The graft-derived cells also express *Pitx2*, *Pax6*, *Msx1/2*, *Pax3*, and *Brn3a*, which are cranial placode markers in Hox-negative regions. When we superadded FGF8 into the medium instead of FGF2, the graft transformed into the neural crest cells. Addition of FGF8 into the medium on condition specific for producing the pre-placodal ectoderm proved ineffective. The epithelial-like cells, therefore, are a precursor to the neural crest and the pre-placodal ectoderm in Hox-negative regions, and they can conditionally be formed upon BMP4 and FGF2. The neural plate cultural system would help clarify the complex array of genes involved in the programming of the placode-specific identity.

Study of the diaphragm: Development and acquisition

The diaphragm is a muscular membrane present only in mammals. The diaphragm separates the mammalian body cavity into the thoracic cavity and the abdominal cavity and plays an important role in respiration. How the diaphragm develops is not clearly understood, and research into its development would be useful for clarifying the cause of congenital diaphragmatic hernia. We marked muscle cells present in the diaphragm and tried to understand how muscle precursor cells migrate into the diaphragm and the timing of cell differentiation. In mouse embryo on embryonic day (E) 10.5, the muscle precursor cells were detected from the upper part of the forelimb to the region of the heart. On E12.5, muscle precursor cells had invaginated into a primitive diaphragm. On E14.5, muscle cells were observed through the diaphragm, as is shown in textbooks of mouse development. Muscle differentiation started from the dorsolateral side of the muscle precursor and had been completed by E14.5. These results might be important for our future studies to understand the development of the diaphragm.

Publications

Kuwahara H¹, Horie T¹, Ishikawa S¹, Tsuda C¹, Kawakami S¹, Noda Y¹, Kaneko T¹, Tahara S¹, Tachibana T, Okabe M, Melki J², Takano R¹, Toda T¹, Morikawa D¹, Nojiri H¹, Kurosawa H³, Shirasawa T¹, Shimizu T¹ (¹Tokyo Metropolitan Inst Gerontol, ²Biol Mol Cell, ³Juntendo Univ). Oxidative stress in skeletal muscle causes severe disturbance of exercise activity without muscle atrophy. Free Radic Biol Med 2010; **48:** 1252-62.

Murata Y¹, *Tamura* M¹, *Aita* Y¹, *Fujimura* K¹, *Murakami* Y², *Okabe M*, *Okada* N¹, *Tanaka* M¹ (*'Tokyo Inst Tech*, ²*Ehime Univ)*. Allometric growth of the trunk leads to the rostral shift of the pelvic fin in teleost fishes. *Dev Biol* 2010; **347**: 236-45.

Department of Molecular Physiology

Shigeru Takemori, Professor

Maki Yamaguchi, Assistant Professor

General Summary

Our efforts have been concentrated on clarifying mechanisms for achieving biological functions from the viewpoint of the cooperative interaction of water and proteins.

Research Activies

Utilization and disorders of water states in the vertebrate locomotive system

The locomotive systems of terrestrial animals should be strong enough to support posture against gravity and compliant enough to enable economical movement. To extract general strategies of vertebrate locomotive systems, we analyzed cartilage and skeletal muscle and evaluated their water state by the use of a nuclear magnetic resonance (NMR)/ magnetic resonance imaging (MRI) technique that observes the relaxation process of tissue water protons. Tissue water in both cartilage and skeletal muscle could be grouped into several components on the basis of characteristic relaxation rates. Functional modulation of the tissues accompanied significant changes in the distribution of tissue water among the components without marked changes in the characteristic relaxation properties of each water component. Healthy cartilage of humans and pigs exhibited gradual distribution of the water fraction with slower relaxation rates: smaller in the layers near the bone surface and larger at the synovial surface. Because water with the slower relaxation rates has been shown to be more strongly restricted by the structural proteins, this gradual distribution is consistent with the mechanical robustness of the cartilage near the bone surface and smooth gliding at the articular interfaces. We believe that both cartilage and skeletal muscle utilize the water state to realize their specific functions in locomotive systems.

Effect of pressure caused by subjects' weight on MR images

The transverse relaxation process of muscle tissue as shown with MRI is a powerful indicator of muscular activity in the field of rehabilitation and sports medicine. However, the factors that affect the transverse relaxation process of muscle tissue are poorly understood.

For example, MR images are normally obtained with the subjects lying on their backs, but the effect of the pressure caused by their weights on MR images of the superficial muscles, such as the gluteus maximus and the soleus, has not been investigated in detail. To clarify this point, we acquired transverse relaxation processes of superficial muscles and deep muscles with MRI and analyzed the results on the basis of our multiexponential model for the water state in muscle tissue established by NMR measurements of dissected muscle tissue from animals.

In both types of muscles, the transverse relaxation processes were successfully classified

into 2 exponentials with the characteristic time constants. In one of the superficial muscle (soleus) with a longer time constant, the relative amplitude gradually increased after the subjects lay on their backs. Because disturbing venous return with a rubber band did not significantly increase the relative amplitude, an unknown mechanism, other than the reasonable increase in the extracellular water volume caused by the weight of subjects, is likely to be involved.

Water activity in the myofilament lattice evaluated by means of specific gravity measurement

To evaluate the water activity within myofibrils, the specific gravity of myofibril suspensions from rabbit psoas muscle was measured in the presence or absence of polyethylenglycol. If polyethylenglycol does not penetrate the myofilament lattice, the specific gravity of the supernatant after centrifugation of the myofibril suspension should be greater than that of the myofibril suspension before penetration. Polyethylenglycol was found to diffuse into the myofilament lattice at half of the external concentration, indicating that the water activity within the myofilament would be different from that of the bulk water.

Structure of troponin mutant studied by X-ray diffraction

An E244D troponin T (TnT) mutant that causes hypertrophic cardiomyopathy is known to increase maximal tension of cardiac muscle fiber (Ohtsuki et al., 2003). To clarify how this mutation enhances the capacity for tension development, we performed X-ray diffraction experiments with skinned muscle fibers in which endogenous troponin had been replaced with wild-type or E244D TnT at BL15A at the Photon Factory (High Energy Accelerator Research Organization, Tsukuba).

The characteristics of the troponin reflections in both fibers did not differ, suggesting similar structural arrangement of E244D and wild-type TnT on thin filaments. The transition from resting to contracting states accompanied a larger change in the intensity ratio of equatorial reflection (1,1/1,0) in E244D fibers than in wild-type fibers, indicating that a larger fraction of myosin heads are recruited for contractile interaction in E244D fibers.

These results suggest that the rearrangement of local electrostatic bonding triggered by the mutation brings about abnormal interactions between TnT and tropomyosin to cause a larger shift of tropomyosin. This larger shift of tropomyosin would allow increased recruitment of myosin heads to interact with actin and would enhance tension-development capacity in the E244D mutant.

Department of Cell Physiology

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

General Summary

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

Research Activities

Depressed length-dependent activation in left ventricular muscles from a mouse model of dilated cardiomyopathy

Cardiac sarcomeres produce greater active force in response to stretch, forming the basis of the Frank-Starling mechanism of the heart. We have demonstrated that length-dependent activation is coordinately regulated via thin filament "on-off" switching and titinbased lattice spacing reduction. In the present study, we investigated how length-dependent activation is altered in a knock-in (KI) mouse model of inherited dilated cardiomyopathy (DCM) with a deletion mutation $\Delta K210$ in the cardiac troponin T gene (TNNT2). Skinned muscle preparations with a diameter of $\sim 100 \,\mu\text{m}$ were obtained from left ventricular papillary muscles of KI and wild-type (WT) mice. An increase in sarcomere length from 1.9 to 2.2 µm shifted the midpoint (pCa₅₀) of the force-pCa curve leftward by ~ 0.2 pCa units in WT muscles. In KI muscles, Ca²⁺ sensitivity was lower (as in Circulatory Research, 2007), and the sarcomere length-dependent shift of pCa₅₀, i.e., ΔpCa_{50} , was less pronounced, with a mean value of ~ 0.1 pCa units. The ΔpCa_{50} , as well as Ca²⁺ sensitivity, became insignificant between WT and KI muscles following thin filament reconstitution with the identical troponin complex (extracted from rabbit fast skeletal muscle). It was also found that at a similar level of protein kinase A-dependent phosphorylation of sarcomere proteins (e.g., troponin I), length-dependent activation was still less pronounced in KI muscles than in WT muscles. The finding that only N2B titin is expressed in both WT and KI muscles suggests that the depressed length-dependent activation in KI muscles is coupled with a change in thin filament "on-off" regulation.

Microscopic analysis of spontaneous sarcomeric oscillations in neonatal cardiomyocytes We have previously demonstrated that cardiac sarcomeres show spontaneous rhythmic oscillations (SPOCs) under partial activation states, namely, at pCa ~6.0 (Ca-SPOC), or at the coexistence of MgADP and Pi under the relaxing conditions (ADP-SPOC). We have reported that the period of SPOCs (both Ca-SPOC and ADP-SPOC) in skinned myocardium correlates with that of the resting heart rate in various animal species (Sasaki, D. et al., 2005 and 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 Ca-SPOC occurred at pCa \sim 6.0 with a frequency of \sim 3 Hz after treatment with ionomycin. The measurement of intracellular Ca²⁺ with fluo-4 confirmed that Ca²⁺ oscillations did not occur under our experimental conditions. As found in adult cardiomyocytes, the sarcomeric oscillations consisted of quick lengthening and slow shortening during Ca-SPOC. It was also found in untreated neonatal myocytes that 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 owing 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 auto-oscillatory properties of cardiac sarcomeres may be involved in the regulation of cardiac beat.

Real-time imaging of single sarcomeres in the rodent heart

Numerous studies have been performed in tissues and cells to clarify the molecular mechanisms of myocardial contraction. However, because of a number of differences between in vitro and in vivo conditions, the dynamics of myocardial sarcomere contractions in living animals is not yet understood. We developed a novel system allowing us to conduct real-time imaging of single sarcomeres in the beating heart in vivo. First, to estimate the magnitude of regional myocardial movement, we performed imaging of fluorescent microspheres (diameter, $\sim 1 \,\mu m$) on the left ventricle of the beating heart of the anesthetized rat. We found that the movement of fluorescent microspheres on the heart took the form of an ellipse, with the major axis of 100 to 200 μ m, when blood pressure and heart rate were within the normal range. Then, we caused green fluorescent protein to be expressed at sarcomeric Z-discs (α -actinin) by using the adenovirus vector system in adult rats and mice and performed real-time imaging of the movement of single sarcomeres at a video rate (30 frames per second) under fluorescence microscopy. Sarcomere length was found to be $\sim 2.00 \,\mu\text{m}$ in the isolated heart of the rat when perfused with Tyrode's solution containing 30 mM 2,3-butanedione monoxime (hence, during diastole). This value is close to what was obtained previously by others under similar experimental conditions using various experimental techniques, i.e., X-ray diffraction and twophoton imaging. We also found that sarcomere length was ~ 1.7 and ~ 2.2 µm during contraction and relaxation, respectively, in the isolated heart of the mouse. Experiments are now underway to visualize the movement of single sarcomeres in the beating heart of the anesthetized mouse.

Ca^{2+} handling and contraction in cardiac papillary muscles with interstitial fibrosis

Cardiac fibrosis is a maladaptive response to pathophysiological conditions, such as cardiac hypertrophy and ischemic heart diseases. However, the changes in Ca^{2+} handling and contraction in the cardiac muscle with interstitial fibrosis remains unclear. We created pulmonary artery banding (PAB) rats for a model of cardiac hypertrophy. Four weeks after operation, the right ventricular weight of PAB rats was significantly greater than that of control rats, indicating right ventricular hypertrophy. Right ventricular papillary muscles of the PAB rats were divided into the interstitial fibrosis group and the nonfibrosis group, to compare with the control group using Masson Trichrome stain. To measure tension with the intracellular Ca^{2+} transients, we used the aequorin method. The peak Ca^{2+} in both the interstitial fibrosis group and the nonfibrosis group was significantly higher than that in the control group. However, peak tension in the interstitial fibrosis group was significantly smaller than that in the nonfibrosis group or the control group. The time to peak Ca^{2+} in the interstitial fibrosis group was significantly longer than that in the nonfibrosis group or the control group. The impairment of tension development in cardiac muscle with interstitial fibrosis is believed to be due to a decrease in the Ca^{2+} sensitivity and a disturbance of the Ca^{2+} release mechanism. However, asynchronous activation of each cardiac myocyte in the fibrotic preparation, due to scarce cellto-cell communication, might be another mechanism.

Collaborative works with the Department of Cardiology

We attempted to observe the effects of endothelin-1on L-type Ca^{2+} channels and its mechanisms. Endothelin-1 expresses its effects through ET_A receptors, and protein kinase-C and Ca^{2+} /calmodulin kinase II are involved in this signal transduction. The Ca^{2+} leak from sarcoplasmic reticulum (SR) is enhanced by stimulation of the sympathetic nervous system through the protein kinase A-dependent phosphorylation of ryanodine receptors. However, FKBP12.6, which modifies ryanodine receptors, is not involved in this process.

The mechanism of dilated cardiac myopathy was investigated, and an angiotensin type 1 antagonist improved the prognosis and cardiac functions. However, the molecular mechanisms have not been clarified, although phospholamban and the phosphorylation of ryanodine receptors were not directly related.

Publications

Irving **T**¹, *Wu* **Y**², *Bekyarova* **T**¹, *Farman GP*¹, *Fukuda N*, *Granzier H*² (*Illinois Inst Tech*, ²*Univ Arizona*). Thick filament strain and inter-filament spacing in passive muscle: effect of titin-based passive tension. *Biophys J* 2011; **100**: 1499-508.

Song X¹, Kusakari Y, Xiao CY¹, Kinsella SD¹, Rosenberg MA¹, Scherrer-Crosbie M¹, Hara K², Rosenzweig A¹, Matsui T¹ ('Harvard Med Sch, ²Kobe Univ). mTOR attenuates the inflammatory response in cardiomyocytes and prevents cardiac dysfunction in pathological hypertrophy. *Am J Physiol Cell Physiol* 2010; **299**: C1256-66. Terui T, Shimamoto Y¹, Yamane M¹, Kobirumaki F, Ohtsuki I, Ishiwata S¹, Kurihara S, Fukuda N ('Waseda Univ). Regulatory mechanism of length-dependent activation in skinned porcine ventricular muscle: role of thin filament cooperative activation in the Frank-Starling relation. *J Gen Physiol* 2010; **136**: 469-82. *Komukai K, O-Uchi J, Morimoto S, Kawai M, Hongo K, Yoshimura M, Kurihara S.* Role of Ca²⁺/calmodulin-dependent protein kinase II in the regulation of the cardiac L-type Ca²⁺ current during endothelin-1 stimulation. *Am J Physiol Heart Circ Physiol* 2010; **298:** H1902–7.

Reviews and Books

Fukuda N, Terui T, Ishiwata S, Kurihara S. Titin-based regulations of diastolic and systolic functions of mammalian cardiac muscle. *J Mol Cell Cardiol* 2010; **48:** 876-81.

Mizuno J¹, *Morita S¹*, *Otsuji M²*, *Hanaoka K³*, *Kurihara S (¹Teikyo Univ, ²Univ Tokyo, ³JR Tokyo Gen Hosp)*. Evaluation of cardiac and myocardial inotropism and lusitropism using halflogistic curve-fitting (in Japanese). *Masui* 2010; **59:** 422-31.

Department of Biochemistry

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

Research Activities

Cancer research

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

2. 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 many tumor cells tested. The mechanism of action of GSH-DXR is 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. This year, the cytotoxic effects of GSH-DXR-encapsulated polymeric micelles were examined. The GSH-DXR-encapsulated micelles exhibited potent cytotoxicity against cancer cells. Further study will be performed to prepare a polymeric micelle labeled with an anti-CD147 antibody for tumor targeting chemotherapy.

3. Six cell lines with epoxomicin resistance were established. The epoxomicin-resistant cell lines are a reliable tool for 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. In an epoxomicin-resistant human endometrial carcinoma cell line, Ishikawa variant, E-cadherin gene (*CDH1*) expression was suppressed *via* overexpression of zinc finger E-box-binding homeobox (ZEB) 1, a transcriptional repressor of E-cadherin. Treatment of parental Ishikawa cells with epoximicin immediately induced ZEB1, followed by transient suppression of E-cadherin expression.

4. The incidence of ovarian clear cell adenocarcinoma is higher in Japan than in North America or Europe. To develop new therapeutic strategies and to more effectively administer current treatments, HAC2 cells were used to examine the relation between intracellular glycogen accumulation, a conspicuous feature of ovarian clear cell adenocarcinoma; the expression of hypoxia-inducible factor (HIF) 1, the main regulator of cellular metabolism in hypoxia; and the exhibition of chemoresistance under such conditions. Expression of several proteins induced by hypoxia, such as HIF1alpha and HIF1 downstream targets, containing glucose transporter isoform1, hexokinase II, pyruvate dehydrogenase kinase 1, and MCT4, were significantly elevated. Hypoxia also induced glycogen accumulation in the cells.

Other research

1. Pharmaceutical plasma products play important roles in controlling many disorders in clinical medicine. However, plasma products are associated with many risks, such as known and unknown infections, because they are usually produced from donated blood. It is an important to supply such pharmaceutical plasma products safely and in sufficient quantities. Our project to produce a large quantity of high-quality human albumin and fibrinogen using a well-defined human hepatocyte cell line cultivated in a radial flow bioreactor has been successful. Concanavalin A affinity-crossed immuno-electrophoresis showed the microheterogeneity of N-linked sugar chains in fibrinogen.

2. Using methods to purify and identify ubiquitinated proteins in biological materials, several ubiquitin-protein conjugates in Tris-saline-soluble and Tris-saline-insoluble but 2% sodium dodecylsulfate-soluble fractions were analyzed from cadmium-exposed human proximal tubular HK-2 cells. Treatment of HK-2 cells with a sublethal concentration of cadmium induced augmentation of water-insoluble but sodium dodecylsulfatesoluble ubiquitin-protein conjugates and insolubilization of the transcription factor signal transducer and activator of transcription (STAT) 6 and, thus, a decrease in normal mole-To clarify the molecular mechanism of cadmium toxicity to renal tissue, a mouse cules. model of cadmium nephropathy was established in which dose-dependent pathological lesions develop in the renal cortex, and the ubiquitin-protein conjugates in renal tissues were analyzed. Large amounts of the weakly soluble ubiquitin-protein conjugates were produced by cadmium treatment in a dose-dependent manner. Cadmium-induced structural changes in renal STAT6 molecules were also observed. These results support the findings of previous cell culture studies, and the structural damage of cellular proteins, which is caused by cadmium exposure, seem to be involved in the expression of renal cellular toxicity in vivo.

Publications

Saito R, Ishii Y, Ito R, Nagatsuma K, Tanaka K, Saito M, Maehashi H, Nomoto H, Ohkawa K, Mano H, Aizawa M, Hano H, Yanaga K, Matsuura T. Transplantation of liver organoids in the omentum and kidney. *Artif Organs* 2011; **35**: 80-3.

Iwase T, Uehara Y, Shinji H, Tajima A, Seo H,

Takada K, Agata T, Mizunoe Y. Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. *Nature* 2010; **465:** 346-9.

Suzuki N, Yachiguchi K, Hayakawa K, Omori K, Takada K, Tabata JM, Kitamura K, Endo M, Wada S, Srivastav AK, Chowdhury VS, Oshima Y, Hattori A. Effects of inorganic mercury on osteoclasts and osteoblasts of the goldfish scales in vitro. J Fac Agr Kyushu Univ 2011; 56: 47-51.

Department of Molecular Biology

Senya Matsufuji, Professor Noriyuki Murai, Assistant Professor Akihiro Oguro, Assistant Professor

General Summary

Polyamines (putrescine, spermidine, and spermine) are ubiquitous biogenic amines that bind to nucleic acids and play essential roles in DNA replication and gene expression. They are also involved in various cellular processes, such as apoptosis, autophagy, and control of ion channels. Cellular polyamine contents are maintained by a feedback mechanism involving the key regulatory proteins antizymes (AZs). AZs are expressed by translational frameshifting in a polyamine-dependent manner and negatively regulate cellular polyamines. AZ is widely conserved in eukaryotes. In mammals there are 3 AZ isoforms (AZ1-3). AZs are further regulated by proteins termed antizyme inhibitors (Azins). Our goal is to clarify the mechanism and biological significance of the elaborate regulatory system and to develop polyamine-related research tools.

Research Activities

Protective effect of AZ1 against over-intake of polyamines

Because polyamines are essential for normal cell function and because the polyamine content in tissues declines with age, polyamines have been evaluated as possible antiaging agents. On the other hand, excess polyamines are known to be harmful. We have shown that elevated levels of putrescine in AZ1-knockout mice disturb ontogenesis and hematopoietic cellular differentiation. To investigate the potential protective role of AZ1 against over-intake of polyamines, we performed a long-term feeding experiment. Adult AZ1-deficient mice and control mice were fed composite meals containing a high dose of polyamines (25 times that of normal meal) for 1 year. At the end of the experiment, animal tissues were subjected to biochemical or morphological examinations. Although no difference was found in body weight or life span between the groups, multiple tumors developed in the livers of only AZ1-deficient mice. The result suggests that AZ1 plays a protective role against tumorigenesis induced by over-intake of polyamines.

Mechanism and significance of c-Myc degradation mediated by AZ2

AZ binds to ornithine decarboxylase (ODC), a key enzyme for polyamine biosynthesis, triggering degradation of the enzyme by proteasomes. It has also been reported that AZ1 binds to and destabilizes various proteins other than ODC. Last year we found that AZ2 binds to c-Myc and accelerates its degradation in cultured cells. We further investigated the mechanism and biological significance of AZ2-mediated c-Myc degradation. Antiproliferative signals are known to accelerate proteasomal degradation of c-Myc via a mechanism that requires phosphorylation at 2 amino acid residues, threonine

58 and serine 62, and subsequent polyubiquitination of c-Myc by a ubiquitin ligase termed F-box and WD repeat domain-containing 7 (FBW7). To characterize the AZ2-mediated c-Myc degradation, we used a mutant c-Myc in which the 2 residues are substituted with alanine (T58A/S62A). In cultured cells, the T58A/S62A mutant was destabilized by coexpressed AZ2, but not by FBW7, suggesting that AZ2-mediated c-Myc degradation is independent of polyubiquitination. The significance of AZ2-mediated c-Myc degradation is mediated, at least in part, by an ubiquitin-independent pathway. We observed that c-Myc degradation was accelerated by UV irradiation and that the acceleration was suppressed by knockdown of AZ2 through RNA interference. Thus, AZ2 likely mediates c-Myc degradation with certain stresses, such as UV irradiation.

Fluorescent visualization of cancer cells by monitoring intracellular polyamines

Cancer cells generally contain elevated levels of polyamines. We are developing a novel method to visualize cancer cells by combining the polyamine-dependent frameshift mechanism of AZ, an endogenous cellular polyamine sensor, and the fluorescent protein techniques. The frameshift signal sequence of AZ1 messenger (m) RNA, either single or in tandem, was inserted between 2 fluorescent proteins, enhanced cyan fluorescent protein (ECFP) and Keima-Red, so that the ECFP-Keima-Red fusion protein was synthesized only under high-polyamine conditions. The constructs were introduced into mammalian cultured cells and analyzed with fluorescent microscopy. We observed fluorescence of both ECFP and Keima-Red in the transfected cells, but the intensity of Keima-Red fluorescence did not correspond to the polyamine concentration. The system should be improved by utilizing more appropriate AZ1 frameshift signals to gain sensitivity to polyamines.

Expression analysis of Azin1

Azin1 is a homolog of ODC that positively regulates cellular polyamines by binding to and inhibiting AZs. Cellular levels of Azin1 change with growth stimuli or tumorigenesis and are regulated by polyamines. To clarify the mechanism of transcriptional regulation of Azin1, we analyzed various transcripts caused by alternative initiation and splicing in both wild-type mice and homozygous *Azin1* gene trap mice. The gene trap mice have a partially lethal condition with decreased tissue levels of ODC and putrescine. However, Northern blotting detected a certain level of Azin1 mRNA remaining in the homozygous gene trap mice, and Western blotting analysis revealed that embryonic fibroblasts from the homozygous gene trap mice contained Azin1 protein at a level 20% to 30% of that in wild-type controls. These results raised the possibility that alternative forms of Azin1 mRNA are transcribed to skip the trapping insertion. Therefore, we determined transcriptional start sites (TSSs) of *Azin1* gene using the oligo-capping method and the reverse transcriptase-polymerase chain reaction with primers designed after the TSS database. The analyses revealed that the *Azin1* gene trap mice contain a small amount of the full-length transcript and a large amount of a transcript lacking exon 2, both starting from the authentic TSS. In addition, the gene trap mice express alternative transcripts with exon 3, which are minimally utilized in wild-type mice. The expression of the transcripts with exon 3 showed a tissue-specific pattern. Furthermore, other forms of alternative spliced transcripts were detected in both wild-type mice and gene trap mice. Some of the transcripts did not contain translational regulatory elements of polyamines or were expected to encode a form of *Azin1* lacking the AZ-binding domain. These results, together with previous findings, demonstrate the complicated regulation of *Azin1* expression.

Development of a cancer diagnostic system with RNA aptamer for polyamines

RNA aptamers are functional RNAs that bind to their target biomolecules with high affinity and can distinguish similar chemical structures. We aimed to develop a simple diagnostic system for cancer using RNA aptamers for polyamines, which are promising biomarkers for cancer. Last year we isolated an RNA aptamer with high affinity for spermine (spermine aptamer). To determine the binding specificity of the spermine aptamer, we compared the affinity of the aptamer to spermine and related amines with the systematic evolution of the ligand exponential enrichment (SELEX) method. The spermine aptamer was shown to have specificity to linear tetra-amines that contain primary amines at both ends and 3 or 4 carbon atoms between amine moieties. Next, we made an affinity column with immobilized spermine aptamer and performed a semiquantitative analysis of spermine in the solution. It detected spermine with a range of 0.01 to 3 mM. In addition, the spermine aptamer was labeled with fluorescein and used to visualize spermine in the solution under UV light.

Publications

Murai N, Murakami Y, Matsufuji S. Protocols for studying antizyme expression and function. *Methods Mol Biol* 2011; **720:** 237-67.

Reviews and Books Ivanov IP (Univ Col Cork), Matsufuji S. Autoregulatory frameshifting in antizyme gene expression governs polyamine levels from yeast to mammals. In: Atkins JF, Gesteland RF, editors. Recoding: Nucleic acids and molecular biology 24. New York: Springer; 2010. p. 281-300.

Department of Pharmacology

Toshihiko Momiyama, Professor Yuji Ohno, Assistant Professor Taro Ishikawa, Assistant Professor Naofumi Kimura, Professor Haruhisa Nishi, Assistant Professor

General Summary

The research interests of the Department of Pharmacology include:

1) Synaptic transmission and its modulation in the basal ganglia and basal forebrain (Toshihiko Momiyama)

2) Respiratory neural activities in *Xenopus* (Naofumi Kimura)

3) Design of secretory proteins (Yuji Ohno)

4) Analysis of the mechanisms underlying histamine release from human-derived mast cells (Haruhisa Nishi)

- 5) High-frequency firing of cerebellar mossy fibers (Taro Ishikawa)
- 6) The basic mechanism of a ketogenic diet: purinergic autocrine regulation of CA3 pyramidal neurons (Masahito Kawamura)

7) Visual response in the cerebellar paraflocculus (Misa Shimuta)

Research Activities

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

Slice-patch-clamp electrophysiological studies were performed to analyze synaptic transmission, its modulation by neuromodulators, and their developmental change in the nigrostriatal or mesolimbic dopaminergic system and the cholinergic system of the basal forebrain. These systems are involved in various psychological functions and their disorders, including Parkinson disease and Alzheimer disease. The regulation of output from these systems to the cerebral cortex was also studied.

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

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

Respiratory neural activities in Xenopus

Aquatic pipid frogs have several 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 the 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 the cranial nerves V and X, the hypoglossal nerve, and the third spinal nerve innervating the "diaphragm." The buccal ventilation-like activity occurred in the 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 31 was secreted from human embryonic kidney cells when the protein was obligatorily expressed in cells transfected with a mammalian expression plasmid with the 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, N-terminal sequences of interleukin 31 from signal peptides to the first glycosylation site (SG sequences) could be crucial. Furthermore, we examined the fusion proteins of p53, which has nuclear localization signal sequences with SG sequences, and aquaporine, which is a membrane protein with SG sequences. We were able to design some secretory proteins associated with SG sequences.

Analysis of the mechanisms underlying histamine release from human-derived mast cells To further study the function of extracellular purines and their purinergic receptors and the effects they may have on type I allergies, LAD2, a human mast cell-derived cell line, was introduced and successfully cultured in our laboratory. LAD2 cells were used in the following types of experiments: studies of cell activation by definitive antibodies and antigens, detection of messenger RNA for purinergic receptors, pharmacological assays of the effects of extracellular purines on both mast cell products (histamine and beta-hexosaminidase) and intracellular Ca²⁺ mobilization, and RNA interference studies of knockdown procedures of intracellular proteins using originally constructed short hairpin RNA plasmids.

The results of these experiments suggest that activation of the Fc ϵ RI (allergic stimulation) in LAD2 cells followed by increases in intracellular Ca²⁺ is enhanced by activation of phosphoinositide 3 kinase. This finding suggests a role for purinergic receptors in allergic stimulation in LAD2 cells.

High-frequency firing of cerebellar mossy fibers

Somatosensory signals from the facial area of rodents are delivered to the cerebellum via pontocerebellar and trigeminocerebellar pathways. Projection fibers of these pathways form mossy fibers, which terminate in the granule cell layer of the cerebellar cortex. It has been previously reported that somatosensory stimulation to the whiskers and the perioral skin triggers burst firings of the mossy fibers and that the instantaneous frequency of such burst action potentials can exceed 700 Hz. What is not known, however, is which of the cerebellar afferent pathways conducts such high-frequency firings. Therefore, we investigated firing properties of projection neurons of the pontine nuclei (PN) and the tri-

geminal nuclei (TrgN) in acute slice preparations. During depolarizing pulses, PN neurons did not fire the high-frequency action potentials but showed regular firings with moderate accommodation. The maximum instantaneous frequency of individual PN cells did not exceed 700 Hz. In contrast, TrgN neurons showed burst firing, which had a high instantaneous frequency exceeding 700 Hz in a subset of cells. These results suggest that the high-frequency firings of cerebellar mossy fibers are direct signals from the TrgN but not from the PN.

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

A ketogenic (low-carbohydrate high-fat) diet has been used successfully to treat pediatric and medically refractory epilepsy. The mechanisms underlying the success of ketogenic diet therapy, however, are not well understood. A ketogenic diet has been reported to increase ATP concentration in the central nervous system and causes mild hypoglycemia. To clarify the role of extracellular purines underlying the anticonvulsant effect of the ketogenic diet, whole-cell voltage clamp recordings were made from CA3 pyramidal neurons in acute hippocampal slices of rats. Under conditions of reduced extracellular glucose and high intracellular ATP concentrations, CA3 pyramidal neurons hyperpolarize themselves via direct ATP release through pannexin-1 channels with the subsequent activation of adenosine A_1 receptors. This autocrine regulation might be an important mechanism underlying the success of a ketogenic diet.

Visual response in the cerebellar paraflocculus

Our previous studies have revealed that the cerebellar paraflocculus receives remarkable visual signals and that around 80% of the granule cells in this area respond to visual stimuli. However, responses of the Purkinje cells in this area have not been investigated. Thus, we made recordings from the Purkinje cells of anesthetized rats. The results indicate that visual stimuli trigger a change in the frequency of "simple spikes" of Purkinje cells. We are now investigating what kind of visual stimulation can evoke "complex spikes" of Purkinje cells.

Publications

Momiyama T. Developmental increase in D 1-like dopamine receptor-mediated inhibition of glutamatergic transmission through P/Q-type channel regulation in the basal forebrain of rats. *Eur J Neurosci* 2010; **32:** 579-90.

Sasaki J¹, Kofuji S¹, Itoh R¹, Momiyama T, Takayama K², Murakami H¹, Chida S¹, Tsuya Y¹, Takasuga S¹, Eguchi S¹, Asanuma K¹, Hore Y¹, Miura K¹, Davies EM², Mitchell C², Yamazaki M³, Hirai H³, Takenawa T⁴, Suzuki A⁵, Sasaki T¹ (¹Akita Univ Grad Sch Med, ²Monash Univ, ³Gunma Univ Grad Sch Med, ⁴Kobe Univ Grad Sch Med, ⁵Kyushu Univ). The Ptdlns(3,4)P(2) phosphatase INPP 4A is a suppressor of excitotoxic neuronal death. Nature 2010: 465: 497-501.

Yoshikawa G¹, Momiyama T, Oya S¹, Takai K¹, Tanaka J¹, Higashiyama S², Saito N¹, Kirino T³, Kawahara N¹ (¹Univ Tokyo Grad Sch Med, ²Ehime Univ Grad Sch Med, ³Res Inst Int Med Center Jpn). Induction of striatal neurogenesis and generation of region-specific functional mature neurons after ischemia by growth factors. Laboratory investigation. J Neurosurg 2010; **113**: 835-50.

Reviews and Books

Masino SA¹, Kawamura M Jr, Ruskin DN¹, Gawryluk J², Chen X², Geiger JD² (¹Trinity Coll, ²*Univ North Dakota).* Purines and the anti-epileptic actions of ketogenic diets. *Open Neurosci J* 2010; **4:** 58-63.

Momiyama T. Neurotransmitter and Nrueo-

modulator (in Japanese). In: Barrett KE, Barman SM, Boitano S, Brools HL, Okada Y, editors. supervisor of translation. Ganong's Review of Medical Physiology. 23th ed. Tokyo: Maruzen; 2011. p. 153-75.

Department of Pathology

Hiroshi Hano, Professor Akihiko Sakata, Professor Masahiro Ikegami, Associate Professor Hiroyuki Takahashi, Associate Professor Yukiko Kanetsuna, Assistant Professor Masaharu Fukunaga, Professor Masafumi Suzuki, Associate Professor Satoru Chiba, Associate Professor Koichi Nomura, Assistant Professor Toru Harada, Assistant Professor

General Summary

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

Research Activities

Pathology of the liver

Oxidative stress in alcoholic fatty liver, nonalcoholic fatty liver, and nonalcoholic steatohepatitis in the early and late stages was investigated immunohistochemically. 8-Hydroxydeoxyguanosine was used as a marker of oxidative stress. This stress marker was expressed in fine granular fashion, especially around fat droplets in the cytoplasm, by hepatocytes in the early stage and was expressed in fine to rough granular fashion in the cytoplasm of macrophages in the late stage. This finding indicates that oxidative stress in the late stage is involved in the transition from fatty liver to steatohepatitis.

We continued an immunohistochemical study of the origin of proliferative ductules in cholestatic liver damage caused by obstructive jaundice. On the basis of the results of immunohistochemical studies with cytokeratin (CK) 7, CK19, and hepatocyte antigen, we have concluded that proliferating ductules originate from hepatocytes by direct transdifferentiation. In the present study coexpression of CK7 and hepatocyte antigen was confirmed by immunofluorescence. In the cases of successful biliary drainage we found marked decreases in the numbers of hepatocytes coexpressing CK7 and hepatocyte antigen, cessation of ductular proliferation, disappearance of proliferating ductules, and remaining proliferating ductules strikingly similar to preexisting ductules. Such histologic changes indicate restructuring of the biliary drainage system, that is, the process of self-assembly of histologic structures in a thermodynamic nonequilibrial system.

We continued a study with a histologic reconstruction method of the process of fibrosis in nonalcoholic steatohepatitis. As the extent of fibrosis progressed, especially in the middle stage of fibrosis, it became more and more evident that bandlike fibrous connections between central vein areas in adjacent lobules surrounded the portal tracts. In contrast to the change in centrilobular areas, the portal area showed a tendency to be preserved until this stage. It is also noteworthy that arteries developed strikingly in fibrotic areas around the veins in the middle stage.

Renal pathology

Additional research focusing on pathologic variables was performed to compare the Oxford classification of immunoglobulin (Ig) A nephropathy with the Japanese classification. In this study a Japanese cohort was investigated retrospectively using the Oxford research method. We found that the Oxford classification of IgA nephropathy was not applicable to a Japanese cohort because of the differences in some variables of both classifications. A new international classification is expected to be applicable to a Japanese cohort.

Interobserver reproducibility between 4 Japanese pathologists was assessed by means of intraclass correlation coefficients with reference to the Oxford and the Japanese classifications of IgA nephropathy. Specimens used were from 90 cases with IgA nephropathy from 15 institutions. Good reproducibility was obtained for the histologic severity of the Japanese classification and the Oxford classification.

Cystic renal cell carcinoma was investigated. Lesions with cystic changes occupied the renal cell carcinoma in various proportions up to 100%. Cases in which more than 50% of cystic renal cell carcinoma was occupied by cystic lesions showed no metastasis and a good prognosis.

Age-associated renal histologic changes were investigated. Forty-five kidneys obtained at autopsy were used for morphometric analyses. Results were as follows. The mean density of glomeruli (per cm²) was more than 4,000 in fetuses but then gradually decreased to 10 to 20 in adults in their 20s. The mean diameter of glomeruli was 60 μ m in newborns, 100 μ m in infants, 130 μ m in persons in their 20s, and 160 μ m in persons in their 60s and 70s. The mean ratio of the glomeruli to the cortical area was 0.6% in infants and 0.2% to 0.3% in teenagers and older groups. The average number of tubules was 2,000 per mm² in newborns and 1,000 per mm² in teenagers and older age groups.

Gastrointestinal pathology

Risk factors for the metastasis of esophageal superficial carcinoma to lymph nodes were investigated using 203 surgically resected tumors. Our aim was to expand the application of endoscopic mucosal resection and to avoid subsequent redundant surgical resection. Lymphatic channels were immunohistochemically stained with D2-40, and blood vessels were stained with Elastica-van Gieson stain and immunohistochemical stains for CD31 and CD34. Multivariate statistical analysis showed that both blood vessel invasion and lymphatic invasion, which were diagnosed immunohistochemically, were most strongly correlated with lymph node metastasis. The negative predictive value was 94.6%.

Lung pathology

Central emphysema was investigated with thick histologic sections (50, 70, 100, and 150 μ m) stained with Masson's Trichrome and Elastica-van Gieson to clarify the morphogenesis. Microscopic examination showed the stepwise histologic features of alveolar destruction. In the early stage we recognized solitary fenestrae of various size in an alveolar wall in the center of the acinus. They fused to form larger fenestrae that occupied the whole area of alveolar walls. As the alveolar change progressed, only the

strands containing thick elastic fibers were left in the alveolar area. On the other hand, the same change took place in the alveolar wall attached to the bronchovascular bundles. As a result, the bronchioles came to lose the support of alveolar walls. The lung structure is normally supported by the tension of the elastic and collagen fiber continuum (the fiber system). It is conceivable that the diseased alveolar area gradually changed to cysts owing to both the destruction of the fiber system and the effect of pressure generated by air flow.

Urogenital pathology

Pathologic stage pT1 urinary bladder cancer showed a wide range of histologic features, from microinvasion to widespread submucosal invasion. Cases of pT1 urinary bladder cancer were classified into 3 groups, that is, pT1a, pT1b, and pT1c, according to the depth of invasion. We analyzed the differences among the 3 groups concerning the period of no symptoms, the period of no deterioration, and the survival time. The period of no deterioration was significantly shorter in the pTc group than in the pTa group. We conclude that the subclassification is useful for prognostic evaluation of patients.

Breast pathology

A total of 191 cases of borderline malignancy of breast cancer were investigated with immunohistochemical studies for actin, p63, and CD10. If cells positive for these markers were distributed throughout the entire intraductal proliferative lesion, papilloma was diagnosed. However, if positive cells were localized along the margin of the intraductal proliferative lesion, determining whether the lesion was benign or not was difficult. Further investigation is needed to solve this problem.

Oncology

We have previously clarified that 8q22-23.2 is a candidate chromosomal region involved in the development and metastasis of hepatocellular carcinoma. This time we tried to identify candidate genes within that region of the chromosome. Gene analysis, however, identified no significant mutation in more than 10 candidate genes, including DLC1 and MTUS1. Possible future subjects of investigation to solve the problem are messenger RNA and protein expression involved in gene function.

Other topics

We reported an autopsy case of Fabry disease. Deposition sites of glycolipids were identified with immunohistochemical staining for Gb3. These sites included cardiac muscles and epithelial cells of the renal glomerulus and tubules and endocrine organs, such as the pituitary gland, adrenal gland, and pancreas. The latter had not yet previously been identified as deposition sites. Therefore, further investigation is needed to confirm deposition in the latter sites.

We reported a case of pulmonary meningioma that appeared after an intracranial meningioma had been resected. Both meningiomas were benign (grade 1). We compared our case to similar cases previously reported. On the basis of the clinical course and the morphology of tumors, we concluded that the pulmonary meningioma was not a primary tumor but rather was a metastasis from the intracranial meningioma.

Publications

Yoshimura N, Goda K, Tajiri H, Ikegami M, Nakayoshi T, Kaise M. Endoscopic features of nonampullary duodenal tumors with narrow-band imaging. *Hepatogastroenterology* 2010; **57**: 462-7.

Fukuda T, Akiyama N, Ikegami M, Takahashi H, Sasaki A¹, Oka H², Komori T³, Tanaka Y⁴, Nakazato Y⁴, Akimoto J⁶, Tanaka M⁶, Okada Y⁶, Saito S (¹Niigata Univ, ²Saitama Med Univ, ³Kitasato Univ, ⁴Tokyo Metropolitan Inst, ⁵Gunma Univ, ⁶Tokyo Med Univ). Expression of hydroxyindole-O-methyltransferase enzyme in the human central nervous system and in pineal parenchymal cell tumors. J Neuropathol Exp Neurol 2010; **69:** 498-510.

Matsumoto N, Umezawa T, Sasaki T (Tokyo Med Univ), Nakajima K, Kanetsuna Y, Sasaki H. Clinical and prognostic value of the presence of irregular giant nuclear cells in pT1 ovarian clear cell carcinoma. *Pathol Oncol Res* 2011; **17**: 605-11. Epub 2011 Jan 28.

Yamane T, Uchiyama K, Ishii T, Nakano M, Kanetsuna Y, Okusa T, Tajiri H. Isolated granulomatous gastritis showing discoloration of lesions after helicobacter pylori eradication. *Dig Endosc* 2010; **22**: 140-3.

Wakui S, Muto T, Motohashi M, Kobayashi Y, Suzuki Y, Takahashi H, Hano H. Testicular spermiation failure in rats exposed prenatally to 3,3',4,4',5-pentachlorobiphenyl. *J Toxicol Sci* 2010; **35:** 757-65.

Miki K, Kiba T¹, Sasaki H, Kido M, Aoki M, Takahashi H, Miyakodo K¹, Dokiya T², Yamanaka H³, Fukushima M¹, Egawa S (¹Translat Res Inform Center, ²Saitama Med Univ, ³Kurosawa Hosp). Transperineal prostate brachytherapy, using I-125 seed with or without adjuvant androgen deprivation, in patients with intermediate-risk prostate cancer: study protocol for a phase III, multicenter, randomized, controlled trial. *BMC Cancer* 2010; **10**: 572.

Takeyama H, Takahashi H, Tabei I, Fukuchi O, Nogi H, Kinoshita S, Uchida K, Morikawa T. Malignant neoplasm in the axilla of a male: suspected primary carcinoma of an accessory mammary gland. Breast Cancer 2010; 17: 151-4. Terui K¹, Nakatani Y¹, Kambe M¹, Fukunaga M, Hshiki T¹, Saito T¹, Sato Y¹, Takenouchi A¹, Saito E¹, Ono S¹, Yoshida H¹ ('Chiba Univ). Kaposiform hemangioendothelioma of the choledochus. J Pediatr Surg 2010; 45: 1887-9. Kamoi S¹, Ohaki Y¹, Mori O¹, Fukunaga M,

Kamoi S¹, Ohaki Y¹, Mori O¹, Fukunaga M, Takeshita T¹ (¹Nippon Med Sch). Determining best potential predictor during high-dose progestin therapy for early staged and well-differentiated endometrial adenocarcinoma using semiquantitative analysis based on image processing and immunohistochemistry. J Nihon Med Sch 2011; **78**: 84-95.

Kawauchi K¹, Nawata H¹, Yamagata Y¹, Yaegashi H², Fukunaga M, Moriya T³, Furuya T¹, Sugino N¹, Sasaki K¹ (¹Yamaguchi Univ, ²Tohoku Univ, ³Kawasaki Med Univ). Chromosomal imbalances detected by comparative genomic hybridization provide evidence that HMB-45-negative uterine angiomyolipomas belong to the PEComa family. *Histopathology* 2010; **56**: 974-7.

Fukunaga M, Suzuki K (St.Luke's Int Hosp), Hiruta N (Toho Univ). Cotyledonoid dissecting leiomyoma of the uterus: a report of four cases. *APMIS* 2010; **118**: 331-3.

Reviews and Books

Fukunaga M, Asanuma K, Irie T. Peculiar chondroblastoma involving multiple tarsal bones. *Skeletal Radiol* 2010; **39:** 709–14.

Department of Virology

Kazuhiro Kondo, Professor

General Summary

Human herpesvirus (HHV-) 6 is capable of establishing a lifelong latent infection of their host, is reactivated frequently. We are studying the molecular mechanism of latency and pathogenesis of HHV-6, and find a novel latent protein of HHV-6 which associate with chronic fatigue syndrome (CFS) and mood disorders. We are also attempting to apply HHV-6 and HHV-7 as tools for studying the mechanism of fatigue and as viral vectors for gene therapy.

Fatigue is an indispensable biological "alarm" for avoiding the state of exhaustion that is caused by severe stress and overwork and might also induce a variety of diseases. We have investigated the molecular mechanisms of HHV-6 and HHV-7 reactivation, which is known to be 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 a HHV-6 and HHV-7, which are reactivated and released into the saliva.

Research Activities

Identification of small protein encoded by the intermediate transcript of HHV-6 1 as a novel latent protein of HHV-6 associated with CFS and mood disorders

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

We studied the expression of SITH-1 by examining the prevalence of anti-SITH-1 antibodies in patients with CFS or mood disorders and in healthy persons. Antibodies were detected by means of indirect immunofluorescence and enzyme-linked sorbensorbent assay (ELISA). Next, an open reading frame of SITH-1 was linked downstream of a glial fibrillary acidic protein promoter and was expressed in glial cells of mice with an adenovirus vector. As they matured, the mice were analyzed with the tail suspension test and for prepulse inhibition and locomotor activity.

With the indirect fluorescent antibody method, rates of positivity for anti-SITH-1 antibodies were high in patients with CFS or mood disorders. In addition, the results of ELISA were highly correlated with those of indirect immunofluorescence. In behavioral experiments, 3-week-old SITH-1 mice showed decreased immobility time on the tail suspension test and showed impaired prepulse inhibition. Meanwhile, 5-week-old SITH-1 mice showed a decrease in spontaneous motor activity and an increase in immobility time on the tail suspension test. Therefore, astrocytes exposed to SITH-1 seem to play a major role in the depressive and manic-like behavior of mice. These results suggest that SITH-1 is involved in the onset of mood disorders.

Application of HHV-6 as a gene therapy vector

HHV-6 is a lymphotropic virus that causes mild disease and has thus attracted interest as a viral vector for gene therapy. We have generated a recombinant HHV-6 virus named H6R28. In the present study, we used the recombinant HHV-6 as a gene therapy vector. In H6R28, HHV-6 U2-U8 genes were replaced with therapeutic genes and selection markers. H6R28 and the wild-type virus showed similar levels of viral replication in the phytohemagglutinin-stimulated umbilical cord blood mononuclear cells. On the other hand, replication of H6R28 was significantly impaired in adult human T cells, which is considered advantageous for developing a viral vector.

We generated a replication-defective HHV-6 vector expressing short hairpin RNA driven by a U6 promoter directed against the cellular molecule CD4 and human immunodeficiency virus (HIV)-1 core protein p24 Gag. In adult human T cells infected with the H6R28 expressing short hairpin RNA, fluorescence-activated cell sorter (FACS) analysis showed greater than 90% infectivity in both CD4-positive T lymphocytes and CD8-positive T lymphocytes. In infected cells a 90% decrease in CD4 expression and an 83% decrease in HIV-1 p24 expression were observed.

Replication-defective H6R28 showed a moderate infection rate in mature dendritic cells (DCs), a finding that suggests H6R28 could be used for anticancer DC therapy. In infected DCs, FACS analysis showed the stable expression of HLA-ABC and HLA-DR, which are usually down-regulated in herpesvirus-infected cells. These findings suggest that antigen-presenting capacity was maintained in DCs infected with replication-defective HHV-6. Therefore, we believe that H6R28 shows great promise as a gene therapy vector for acquired immunodeficiency syndrome and cancer.

Molecular mechanism and major cause of fatigue

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

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 produced by 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 more than 10-fold with fatigue induced by forced swimming or sleep deprivation.

Our study describes a novel signal transduction pathway for fatigue and its relationship

with possible fatigue-causing substances, such as cytokines, and oxidative stress.

Reviews and Books

Kondo K. Human HHV-6 and 7: latency, reactivation and diseases (in Japanese). *Kagaku Ryoho no Ryoiki* 2010; **26:** 1211-7.

Kondo K. Virus infection and fatigue (in Japanese). *Anti Aging Igaku* 2010; **6:** 343-7.

Kondo K. Herpesvirus: fatigue and mood disorder (in Japanese). In: Kamihata T, editor. Medicine of fatigue. Tokyo: Nippon Hyoronsha; 2010. p. 170-80.
Department of Bacteriology

Yoshimitsu Mizunoe, Professor

Hitomi Shinji, Assistant Professor

General Summary

Research projects of our department have focused on: 1) the role of fibronectin-binding proteins (FNBP) A and B in staphylococcal infection, 2) the analysis of *Staphylococcus aureus* biofilm formation and detachment, and 3) the molecular mechanisms of bacterial ATP secretion.

Research Activities

Role of FnBPA and FnBPB in in vitro cellular infections and in vivo septic infections by S. aureus

FnBPA and FnBPB are important adhesins for *S. aureus* infection. We constructed strains with mutations of *fnbA* or *fnbB* or both from *S. aureus* SH1000, which possesses intact *rsbU*, and studied the role of these adhesins in *in vitro* and *in vivo* infections. In intravenous infection, all *fnb* mutants caused a marked reduction in the colonization rate and the mortality rate of mice. The *fnbB* mutant caused a more severe decrease in body weight than did the *fnbA* mutant. Serum levels of interleukin 6 and nuclear factor (NF)-κB activation in spleen cells were markedly reduced in *fnbA* or *fnbA/B* mutant infections; however, there was no significant reduction in *fnbB* mutant infections. In *in vitro* cellular infection, FnBPA was shown to be indispensable for adhesion to and internalization by nonprofessional phagocytic cells upon ingestion by inflammatory macrophages and NF-κB activation. However, both FnBPs were required for efficient cellular responses. The results showed that FnBPA is more important for *in vitro* and *in vivo* infections; however, cooperation between FnBPA and FnBPB is indispensable for the induction of severe infection resulting in septic death.

Analysis of biofilm detachment factor secreted by S. aureus

The bacteria within the biofilm matrix are protected from the host immune system and from antibiotic attack. Therefore, finding the biofilm-disassembling substance might prove widely useful in medical and industrial applications for preventing or eradicating biofilms. The biofilm matrix formed by *S. aureus* is composed of protein, polysaccharides, and DNA. We found that *S. aureus* secreted the factor that detaches its own biofilm. The culture supernatant of *S. aureus* also detached the biofilms of *Staphylococcus epidermidis*, methicillin-resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa*, and *Escherichia coli*, which are causative bacteria of biofilm infections. The molecular weight of the factor responsible for the detachment effect is less than 500 Da. On-going study focuses on identifying the factor.

Identification and spatiotemporal dynamics of S. aureus biofilm matrix proteins

All biofilms contain an extracellular matrix that holds cells together. This matrix is often composed of polysaccharide biopolymers along with extracellular DNAs and proteins. Although proteinaceous components of the *S. aureus* biofilm matrix could be targets for vaccine development, they are largely unknown. Here, we surveyed *S. aureus* biofilm matrix proteins with proteomic approaches. Cell surface proteins were extracted from biofilms formed by a clinically isolated MRSA strain and subjected to top-down proteome analysis. A total of 133 proteins, including adhesins required for attachment to host cells, biofilm-related proteins, cell wall-anchored proteins, cytoplasmic proteins, and proteins of unknown function, were identified. A green fluorescent protein reporter assay revealed that MHC class II analog protein (Map), a predominant protein among them, formed extracellular biofilm matrix structures. In addition, purified recombinant Map stimulated biofilm formation by *S. aureus* in a dose-dependent manner, indicating that Map plays a crucial role in *S. aureus* biofilm formation. Roles of the other identified in the staphylococcal biofilm formation proteins are under investigation.

Studies on characteristics of S. epidermidis against S. aureus

Last year we reported 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.

We have addressed the ability of Esp-secreting *S. epidermidis* to prevent colonization by MRSA. MRSA has emerged in recent decades as a leading cause of infections worldwide and predisposes to infection, but available regimens are ineffective at preventing MRSA colonization. Studies of human nasal flora suggest that resident bacteria play a critical role in limiting *S. aureus* growth and prompted us to ask whether application of commensal resident bacteria can prevent nasal colonization with MRSA. We established a murine model system to study this question and showed that nasal precolonization with *S. epidermidis* is enhanced by prior application of streptomycin. Once nasally colonized with *S. epidermidis*, the mice became more resistant to colonization with MRSA. Our study suggests that application of commensal bacteria with antibiotics could represent a more effective strategy to prevent MRSA colonization.

Characterization of ATP-secreting bacteria from mice and humans

We have reported that ATP-secreting bacteria are present in the intestines of mice and humans. However, the mechanisms of ATP secretion in bacteria are not completely understood. To investigate the mechanisms, we have generated mutants that cannot secrete ATP. We are investigating the mechanisms using these mutants.

Publications

Iwase T, Uehara Y^I, Shinji H, Tajima A, Seo H^I, Takada K, Agata T, Mizunoe Y (^IKochi Med Sch). Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. *Nature* 2010; **465**: 346-9.

Iwase T, Shinji H, Tajima A, Sato F, Tamura T, Iwamoto T, Yoneda M (Univ Tokyo), Mizunoe Y. Isolation and identification of ATP-secreting bacteria from mice and humans. *J Clin Microbiol* 2010; **48:** 1949–51.

Kitagawa R¹, *Takaya* A¹, *Ohya* M¹, *Mizunoe* Y, *Takade* A², *Yoshida* S², *Isogai* E¹, *Yamamoto* T¹ (*'Chiba Univ*, ²*Kyushu Univ)*. Biogenesis of Salmonella enterica serovar typhimurium membrane vesicles provoked by induction of PagC. *J Bacteriol* 2010; **192:** 5645-56.

Abdullah-Al-Mahin¹, Sugimoto S, Higashi C¹, Matsumoto S¹, Sonomoto K¹ ('Kyushu Univ). Improvement of multiple-stress tolerance and lactic acid production in Lactococcus lactis NZ9000 under conditions of thermal stress by heterologous expression of Escherichia coli DnaK. Appl Environ Microbiol 2010; **76**: 4277-85.

Piao H¹, Minohara M¹, Kawamura N¹, Li W¹, Mizunoe Y, Umehara F¹, Goto Y¹, Kusunoki S¹, Matsusita T¹, Ikenaka K¹, Maejima T¹, Nabekura J¹, Yamazaki R¹, Kira J¹ ('Kyushu Univ). Induction of paranodal myelin detachment and sodium channel loss in vivo by Campylobactor jejuni DNA-binding protein from starved cells(C-Dps) in myelinated nerve fibers. J Neurol Sci 2010; 288: 54-62.

Piao H¹, Minohara M¹, Kawamura N¹, Li W¹, Matsushita T¹, Yamasaki R¹, Mizunoe Y, Kira J¹ (¹Kushu Univ). Tissue binding patterns and in vitro effects of Campylobacter jejuni DNA-binding protein from starved cells. Neurochem Res 2011;

36: 58-66.

Sato F, Iwase T, Tajima A, Shinji H, Mizunoe Y. Biofilm formation of clinical isolated Staphylococcus species (in Japanese). BACTERIAL ADHEREN & BIOFILM 2010; 23: 23-8.

Reviews and Books

Iwase T, Mizunoe Y. Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization (in Japanese). *Jikken Igaku* 2010; **28:** 2635-8.

Iwase T, Mizunoe Y. Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization (in Japanese). *LUNG* 2010; **18:** 400-4.

Sugimoto S, Sonomoto K (Kyushu Univ). En/ Shintoatsu/Kansou (in Japanese). In: Japan Society for Lactic Acid Bacteria. Nyusankin to bifizusukin no saiensu. Kyoto: Kyoto University Press; 2010. p. 238-40.

Sugimoto S, Sonomoto K (Kyushu Univ). Ondo (in Japanese). In: Japan Society for Lactic Acid Bacteria. Nyusankin to bifizusukin no saiensu. Kyoto: Kyoto University Press; 2010. p. 240-6.

Department of Public Health and Environmental Medicine

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

General Summary

Our major research projects in the 2010 academic year focused on: 1) induction of micronuclei and chromosome aberrations in human peripheral blood lymphocytes from workers exposed to indium tin oxide; 2) examination of the chromosomal aberration test method using the rat peripheral blood lymphocyte; 3) taste disorder induced by zinc deficiency in rat and oxidative stress; 4) mutagenecity caused by hypertension; 5) zinc excess intake enhances the induction of micronuclei in rat bone marrow cells; 6) evaluation of mutagenic potential related to diabetes mellitus; 7) a work of the method for analysis of 8-hydroxydeoxyguanosine (8-OHdG) with gas chromatography/mass spectrometry (GC/ MS); 8) evidence-based medicine (EBM); 9) cost-effectiveness of vaccination; 10) prevalence of menopausal symptoms; 11) associations between body weight, waist circumference, and cardiovascular risk factors; 12) long-term follow-up study in patients with type 2 diabetics; 13) pressure ulcer healing and zinc supplementation with polaprezinc; 14) questionnaire survey of professional divers; and 15) mental health in the workplace.

Research Activities

Experimental Medicine

1. Induction of micronuclei in human peripheral blood lymphocytes from workers exposed to indium tin oxide

Human peripheral blood lymphocytes derived from workers exposed to indium tin oxide were incubated for 48 hours in RPMI 1640 containing phytohemagglutinin. The cells were then cultured with cytochalasin B for an additional 48 hours. The frequency of micronuclei in each worker was 0.7% to 1.7%.

2. Induction of chromosome aberration in human peripheral blood lymphocytes from workers exposed to indium tin oxide

Human peripheral blood lymphocytes from workers exposed to indium tin oxide were incubated for 48 hours in RPMI 1640 containing phytohemagglutinin. The result showed no chromosome aberration attributable to exposure to indium tin oxide.

3. Examination of the chromosomal aberration test method using rat peripheral blood lymphocytes

A study is in progress to establish optimal concentrations of several kinds of mitogen.

4. Taste disorder induced by zinc deficiency in rat and oxidative stress

Rats were fed a zinc-deficient diet with distilled water and hydrochloride quinine. Hydrochloride quinine intake increased in rats fed the zinc-deficient diet. The rats fed the zinc-deficient diet received injections of tempol. Hydrochloride quinine intake did not decrease in rats fed the zinc-deficient diet. The induction of taste disorder in rats with zinc deficiency was not related to the active oxygen species.

5. Mutagenecity caused by hypertension

Hypertension is a lifestyle-related disease in which increased oxidative stress can be excessive, suggesting that cancer risk increases with oxidative DNA damage. The *in vivo* micronucleus test was performed with hypertensive rats aged 28 to 40 weeks. Damage to DNA tended to increase with each passing micronuclei induction. In the future, we will evaluate 8-OHdG.

6. Excess zinc intake enhances the induction of micronuclei in rat bone marrow cells

Zinc is an essential trace element that works as an active center of approximately 300 enzymes to maintain cellular functions. Recently, zinc can be administered with diet supplements and other preparations. However, many people do not abide the adequate dosage by a wrong idea, the promotion of health or more by taking zinc and many supplements. Moreover, zinc toxicity is not widely known. Therefore, we examined mutagenicity due to zinc.

7. Evaluation of mutagenic potential related to diabetes mellitus

Effect of streptozotocin-induced diabetes mellitus on micronucleus formation in rats was studied with the in *vivo* micronucleus assay. Diseased animal was killed at 4, 7, 10, and 14 weeks after induction of diabetes mellitus. A tendency for the micronucleus frequency to increase was observed, but this difference was not significant. A more sensitive method is required to confirm this result.

8. A work of the method for analysis of 8-OHdG by GC/MS

The variable that best indicates the oxidation damage of DNA is 8-OHdG. For this reason, the measurement of 8-OHdG with GC/MS was investigated.

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 two decades. These principles have evolved in tandem with an explosion of epidemiologic activity covering a wide range of health problems. Our greatest concern is to clarify risk factors for adult disease and intractable diseases. We also studied the methodology of medical informatics education and EBM.

2. Cost-effectiveness of vaccination

We established a standard method to analyze the cost-effectiveness of vaccination and assessed the cost-effectiveness of implementing routine vaccination programs in Japan: 6 vaccines for children (*Haemophilus influenzae* type b, pneumococcal conjugate vaccine 7, varicella, mumps, hepatitis B virus, and human papilloma virus) and 1 vaccine for adults (pneumococcal conjugate vaccine 23).

3. Prevalence of menopausal symptoms

Questionnaire surveys on menopausal symptoms were administered to 50- and 60-yearold women who lived in northern Kawasaki. We determined the prevalence of awareness about menopause and symptoms in community-dwelling Japanese women. 4. Associations between body weight, waist circumference, and cardiovascular risk factors

Using annual health examination data, we examined the associations between body weight, waist circumference, and cardiovascular risk factors in Japanese male workers.

5. Long-term follow-up study in type 2 diabetics

Fasting plasma glucose (FPG) variability was a risk factor for nonproliferative and proliferative diabetic retinopathy independent of the mean FPG or HbA1c in patients with type 2 diabetes. The development of proliferative diabetic retinopathy was also significantly associated with mean HbA1c more than 5 years earlier and with mean FPG more than 10 years earlier.

6. Pressure ulcer healing and zinc supplementation with polaprezinc

We performed a nonrandomized controlled clinical trial to examine the effects of the zinccontaining preparation polaprezinc on the healing of chronic pressure ulcers. The results of this trial suggest that polaprezinc is beneficial for the treatment of pressure ulcers.

7. Questionnaire survey for professional divers

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

8. Mental health in the workplace

Mental health in the workplace is increasingly recognized as a serious problem. There are some questionnaires to prevent mental disease in Japan. A concrete question in these questionnaires is the importance of managing stress in the workplace. The purpose of this study was to investigate stress in the workplace with a new questionnaire.

Publications

Miyazaki T, Takenaka T, Inoue T, Sato M, Hanyu M, Eiki Y, Nodera M, Yanagisawa H, Ohno Y, Shibasaki S, Suzuki H. Klotho expression is induced by calorie restriction in adult male rats. *Trace Nutrients Res* 2010; **27**: 92-6.

Iwase T, Uehara Y, Shinji H, Tajima A, Seo H, Takada K, Agata T, Mizunoe Y. Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. *Nature* 2010; **465:** 346-9.

Suka M, Miwa Y, Ono Y, Yanagisawa H. BMI, waist circumference, and clustering of cardiovascular risk factors in Japanese adults. *Environ Health Prev Med* 2011; **16**: 90-6. Takao T, Ide T, Yanagisawa H, Kikuchi M, Kawazu S, Matsuyama Y. The effect of fasting plasma glucose variability on the risk of retinopathy in type 2 diabetic patients: retrospective long-term follow-up. *Diabetes Res Clin Pract* 2010; **89:** 296-302.

Reviews and Books

Takagi R, Suzuki Y, Seki Y, Ikehata M, Kajihara C, Shimizu H, Yanagisawa H. Indium chloride-induced micronuclei in in vivo and in vitro experimental systems. J Occup Health 2011; 53: 102-9.

Department of Forensic Medicine

Kimiharu Iwadate, Professor

Kenji Fukui, Assistant Professor

General Summary

Our main research projects in 2010 have focused on sudden unexpected infant death due to milk aspiration, diagnosis of drowning by detection of specific DNA fragments of aquatic bacteria from blood samples, analysis of the ubiquitin proteasome system and the autophagy lysosome system in the central nervous system, identification of war-dead remains by DNA analysis, the objective evaluation of the limit of DNA typing based on the intensity of ninhydrin treatment, and quantitative analyses of medicines and poisonous substances in forensic autopsy cases.

Research Activities

Forensic pathology

1. Sudden unexpected infant death due to milk aspiration

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

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

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

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

Research associated with the ubiquitin proteasome system and the autophagy lysosome system, which play major roles in the degradation of intracellular proteins and organelles, has recently advanced in various areas of medical science. How the ubiquitin proteasome system and the autophagy lysosome system are induced in traumatic intracranial injury was investigated in cases of such injury examined at autopsy at our department. Both degradation pathways were found to be induced in the injured cortex soon after trauma, and the pathway involved in the degradation of unnecessary substances or the cells in which the degradation is activated may be different or be altered over time

after the traumatic event in the central nervous system.

DNA analysis

1. Identification of war-dead remains with DNA analysis

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

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

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

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/mass spectrometry, and spectrum photometry in tissue specimens obtained at autopsy.

2. Qualitative and quantitative analyses of hydrogen sulfide

We performed autopsies in 17 cases of death due to the inhalation of intentionally generated hydrogen sulfide gas. The concentrations of sulfide and thiosulfate in blood, urine, and cerebrospinal fluid were examined with gas chromatography/mass spectrometry. Although in previous reports of cases of hydrogen sulfide poisoning, the blood concentration of thiosulfate was higher than that of sulfide, in the present study the concentration of sulfide was higher than that of thiosulfate in 9 of 17 cases. Furthermore, the sulfide concentrations were similar to those in previously reported cases of accidental fatal poisoning in 9 of our 17 cases and resembled those in previously reported cases of intentional fatal poisoning in 2 of our 17 cases.

3. Examination of a method for analyzing tandospirone citrate

We detected tandospirone citrate, a serotonergic anxiolytic, in 2 autopsy cases. Qualitative and quantitative methods of analyzing tandospirone citrate with gas chromatography/ mass spectrometry were examined. With quantitative analysis, high concentrations of tandospirone citrate were detected.

Radiocarbon analysis

1. Establishment of age estimation

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

Publications

Sakai K, Takatsu A, Shigeta A, Fukui K, Maebashi K, Abe S, Iwadate K. Potential medical adverse events associated with death: a forensic pathology perspective. Int J Qual Health Care 2010; **22**: 9-15.

Kondo-Nakamura M, Fukui K, Matsu'ura S¹, Kondo M¹, Iwadate K (¹Ochanomizu Univ). Single tooth tells us the date of birth. Int J Legal Med 2010 Oct 17. Epub ahead of print. Sakai K, Maruyama-Maebashi K, Takatsu A, Fukui K, Nagai T, Aoyagi M, Ochiai E, Iwadate K. Sudden death involving inhalation of 1,1-difluoroethane (HFC-152a) with spraycleaner: three case reports. Forensic Sci Int 2011; **206:** e58-61.

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 parasitic infections

Mast cells and basophils are known to induce allergic reactions. We have studied under our own hypothesis that these cells play protective roles against parasites. We have demonstrated that mast cell-derived tumor necrosis factor (TNF) and vascular endothelial growth factor (VEGF) are responsible for protection in malaria. Serum levels of VEGF after Plasmodium berghei infection were lower in TNF-deficient mice than in wild-type mice, indicating that the secretion of VEGF depends on TNF in murine malaria. In addition, VEGF was likely to be involved in the pathogenesis of malaria through heme oxyganase 1. The effect of basophils was examined with new basophil-deficient mice in which differentiation of basophils could be blocked at any time through a signal from diphtheria toxin receptor by gene manipulation. No difference in protection against primary infection with tick Haemahysalis longicornis was found between basophil-deficient mice and wild-type mice. When basophil-deficiency was induced at the time of secondary infection with Haemahysalis longicornis, protective activity was significantly reduced, suggesting that basophils play a protective role against secondary infection with ticks.

Exhaustive analysis of glycosylation alterations of intestinal mucus in mice infected with an intestinal nematode, Nippostrongylus brasiliensis

The mucus that covers mucosal epithelium is home to intestinal parasites and serves as a platform for host-parasite interactions, including establishment and expulsion. The main component of mucus is mucin, although many factors, derived from immune cells or epithelium, are dissolved in mucus. Previously, we suggested that interleukin (IL) 13, a type 2 cytokine, up-regulates message level of sialyltransferases and the number of sialomucin-positive goblet cells through IL-4 receptor/Stat6 signal pathway. We herein exhaustively examined immune-mediated glycosylation alterations of intestinal mucus in mice infected with an intestinal nematode, *Nippostrongylus brasiliensis*. Mucus was washed out of the small intestine on day 9 after infection and analyzed by means of lectin array analysis or mass spectrometry; day 9 is when worms are expelled from the gut. In lectin array analysis, the binding affinity of the mucus to 45 known lectins was measured. Twelve lectins showed greater than a 2-fold increase, whereas 3 lectins showed a

decrease of less than 50% when compared with that of the uninfected mucus. After hydrazine degradation treatment, isolated sugar chains were subjected to matrix-assisted laser desorption ionization-time of flight mass spectometry and analyzed with the Glyco-Mod software tool (http://web.expasy.org/glycomod/). Consistent results of both lectin array and mass spectrometry analyses were increased expression of deoxyhexose (fucose) and conjugated polysaccharides composed of deoxyhexose, hexosamine, and N-acetyl-neuraminic acid (sialic acid) in day-9 mucus. These results support our previous observations and suggest that host immune responses induce glycosylation alterations of the intestinal mucus to serve as a physical barrier to the enforce physiological condition of the intestine to drive out intestine-dwelling parasites.

The mechanism of encystation and excystation in Entamoeba

We examined changes in gene expression of actin and actin depolymerization factor cofilin of *Entamoeba invadens* during differentiation by real-time reverse transcriptase-polymerase chain reaction. First, we identified 3 cofilins (Cfl-1, 2, 3). Actins and Cfl-2 were expressed in trophozoites and were decreased during encystation. During excystation, actins and 3 cofilins were expressed. Contrary to our expectations, we had previously found that cytochalasin D promoted excystation. Accordingly, we found that the expression of Cfl-1 and Cfl-3 was markedly increased in the presence of cytochalasin D. Thus, we demonstrated that Cfl-1 and Cfl-3 are expressed only during excystation and that the enhancement of excystation by cytochalasin D is associated with the increased expression of Cfl-1 and Cfl-3.

Transcriptome analysis of Entamoeba with an ultrafast sequencer

Data from several genes has shown that the 5' untranslated region (5' UTR) of the messenger RNA of *Entamoeba histolytica* is extremely short. However, comprehensive analysis was difficult because the 5' end of complementary DNA tends to be easily missing. Using transcription start site (TSS) sequencing method, which is a combination of the oligocapping method and ultrafast sequencing, we determined an extensive number of short sequences beginning with the TSS from trophozoites of *E. histolytica* and *Entamoeba invadens*. As a result, short 5' UTRs (around 10 base pairs) of these *Entamoeba* species have been comprehensively demonstrated. We also sequenced the RNAs of both amoebas and discovered several new genes that had not been predicted from the genome sequences. The exon-intron structure and alternative splicing were also discovered. Promoter sequences are now under investigation. The sequencing of TSSs and RNAs allows us to analyze the level of gene expression by counting tag numbers. Future research will attempt to identify genes related to encystation by comparing levels of gene expression between the trophozoites and cysts of *E. invadens*.

Resistance against malaria in thalasemia

Malaria is a major killer in tropical area. In particular, malaria caused by *Plasmodium falciparum* is lethal and demands research to prevent or cure the disease. However, *P. falciparum* infects only humans and some nonhuman primates. Therefore, performing research in animals is difficult. To overcome this problem, we are developing a mouse

model that is able to survive with human red blood cells (RBCs) rather than mouse RBCs. To eradicate mouse RBCs, hematopoietic stem cells are taken from embryonic lethal thalassemia model mice, which cannot produce RBCs, and transplanted to lethally irradiated severe combined immunodeficiency mice. If this model is successfully developed, it may facilitate malaria research and provide benefits to many patients.

Publications

Kumagai M, Inaba T, Makioka A, Ishiwata K, Onishi K, Watanabe N. An improved glycerin-jelly mounting procedure for permanent preparations of helminth eggs. *J Parasitol* 2010; **96:** 440-1.

Wada T, İshiwata K, Koseki H, Ishikura T, Ugajin T, Ohnuma N, Obata K, Ishikawa R, Yoshikawa S, Mukai K, Kawano Y, Minegishi Y, Yokozeki H, Watanabe N, Karasuyama H. Selective ablation of basophils in mice reveals their nonredundant role in acquired immunity against ticks. J Clin Invest 2010; **120**: 2867-75.

Makioka A, Kumagai M, Hiranuka K, Kobayashi S, Takeuchi T. Entamoeba invadens: identification of ADF/cofilin and their expression analysis in relation to encystation and excystation. *Exp Parasitol* 2011; **127:** 195-201. *Kumagai M, Katayama T.* Problems on the diagnosis and treatment of cutaneous myiasis due to *Dermatobia hominis* in Japan (in Japanese). *Ohara Sogo Byoin Nenpo* 2010; **50**: 31-6.

Sakuraba R, Tanaka K, Kumagai M, Kida K, Inaba T. Studies on Acanthamoeba spp.—Isolation of the pathogenic Acanthamoeba from sand of the children's play ground and house dust in Hirosaki City—(in Japanese). Hoken Kagaku Kenkyu 2011; **1**: 147-54.

Reviews and Books

Kumagai M, Nishino T. Mefloquine, Fansidar, Quinine, and antimalarial drugs for prophylaxis(in Japanese). Medical Practice 2010; **27:** 1561-4.

Department of Laboratory Medicine

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

General Summary

Research projects in our department in 2010 were concerned with clinical physiology, clinical microbiology, clinical chemistry, hematology, cardiology, clinical cell biology, and clinical psychiatry. Research achievements in each division are described below.

Research Activities

Clinical physiology

This study examined the effects of long-tem caffeine intake and regular exercise, as well as their combination, on muscle triglyceride and glycogen content in the liver and skeletal muscle, an indicator of insulin resistance. Twenty-four Otsuka Long-Evans Tokushima fatty rats, an obese-diabetic animal model, were assigned to a sedentary group, an exercise group, a caffeine group, and a caffeine and exercise group for 5 weeks of treatment and then compared with a group of control rats. The caffeine group and the caffeine and exercise group were fed rat chow containing 0.25% caffeine, and the exercise group and the caffeine and exercise group were fed rat chow containing to exercise every day. The pretreatment and posttreatment levels of serum biochemical components were measured after overnight fasting, and the posttreatment triglyceride and glycogen contents in the liver and quadriceps femoris were measured. Combined long-term caffeine intake and exercise expenditure improves insulin resistance in both the liver and skeletal muscles and metabolic syndrome risk factors in Otsuka Long-Evans Tokushima fatty rats more effectively than does caffeine intake or exercise alone.

Clinical microbiology

Blood stream infection in children was studied with the Multiplex PCR System (Gen-Script USA, Inc., Piscataway, NJ, USA) (in combination with Seeplex sepsis tests [Seegene, Seoul, Korea] and the MultiNA DNA/RNA analysis system [Shimadzu Corp., Kyoto]). This system has the potential to rapidly and easily identify bloodstream infections.

Molecular epidemiologic analysis of *Clostridium difficile* was performed. Most strains were characteristic of toxin A-negative, toxin B-positive variant strains.

Some clinically isolated, previously unidentified bacteria strains were identified though gene sequencing of polymerase chain reaction-amplified 16S ribosomal RNA.

Methods have been improved for the molecular detection and identification of bacteria species from paraffin-embedded tissues.

Clinical chemistry

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

We reported the following study findings.

(1) The significance of very low density lipoprotein (VLDL) cholesterol measurements with our established HPLC lipoprotein analysis method for monitoring the exercise-induced amelioration of lipid metabolism and taking longer time for the improvement of adiponectin and insulin resistance than for the amelioration of VLDL metabolism were verified and published in the *Journal of Atherosclerosis Thrombosis*.

(2) The HPLC method was revised to measure lipoprotein a in a collaborative project and reported in the *Journal of Lipid Research*.

(3) Because of the advantages of this HPLC method, Hiroshi Yoshida was awarded the Life Science Prize at the 56th National Congress of Japanese Society of Laboratory Medicine, and his paper was published in *Rinsho Byori*.

(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 Japanese Society of Laboratory Medicine and was published in *Rinsho Byori* in an article entitled "Frontlines of oxidized lipoprotein research."

(5) Atherosclerosis-related serum lipid markers (remnant lipoprotein cholesterol and small, dense LDL) presented at a symposium of the 41st Annual Meeting of the Japan Society of Clinical Laboratory Automation was published in the *Japanese Journal of Clinical Laboratory Automation*.

(6) The ameliorating effects of astaxanthin on triglycerides, high-density lipoprotein cholesterol, and adiponectin were presented in a workshop at the 15th Congress of the International Atherosclerosis Society (Boston, MA, USA) and were described in a paper published in *Atherosclerosis*.

(7) Subanalysis by sex from findings of the Jikei Heart Study was published in the *Journal of Hypertension*; (8) A review paper entitled "Mechanisms of LDL oxidation" was published in *Clinica Chimica Acta* in response to an invitation from the journal's editor.

2. Successful eradication of *Helicobacter pylori* is extremely important for preventing the progression of gastroduodenal diseases. A triple regimen combining a proton-pump inhibitor (PPI) and 2 antibiotics (clarithromycin and amoxicillin or metronidazole) is now considered the gold standard therapy for eradicating *H. pylori*. However, the eradication rate of first-line treatment is only 70% to 85% and has tended to decrease because of increasing resistance to clarithromycin and metronidazole. In the present study we examined the susceptibility of *H. pylori* to antibiotics and a PPI using *in vitro* determination of the minimum inhibitory concentration (MIC) and the patient's genetic polymorphism of cytochrome P450 2C19 (CYP2C19), an enzyme that metabolizes PPIs, in 40 patients with *H. pylori* infection and dyspeptic diseases in whom first-line eradication therapy had failed. Then we have eradicated such infected *H. pylori* by more suscept-

able triple selected regimen. The rate of successful eradication with second-line or third-line therapy was lower in homo/hetero extensive metabolizers than in poor metabolizers of CYP2C19. The selection of 2 antibiotics determined with the MIC *in vitro* appeared to result high eradication rate.

Hematology

More attention should be paid to hematological neoplasms with the translocation derivative (1;7) (q10;p10).

The translocation derivative (1;7) (q10;p10) is sometimes observed in myeloid neoplasms, but its precise characteristics are unknown. We evaluated 13 patients who had hematological neoplasms with derivative (1;7). Six of the 13 patients had a history of chemotherapy, indicating the importance of secondary malignancy. Most patients were men, and all of the patients with *de novo* disease were men and had a median age of 74 years. Laboratory data revealed that mean corpuscular volume was high, thrombocytopenia was not apparent, dysplasia was common, and complex chromosome abnormalities were not present. The prognosis was not as poor as previously reported: the estimated 3-year-survival rate was 35%. Although the patients were too few for clear conclusions to be made, more attention should be paid to this chromosome aberration.

Cardiology

We are studying catheter intervention for atrial fibrillation. To eliminate atrial fibrillation, we use the technique of segmental ostial catheter ablation (SOCA). We have developed 2 new methods for SOCA. One method is SOCA with a large lasso catheter, and the other is intravenous administration of ATP to induce transient pulmonary vein (PV) reconduction (dormant PV conduction) following PV isolation. This year we revealed the detailed characteristics of dormant PV conduction in patients with atrial fibrillation who underwent catheter ablation.

Clinical cell biology

1. ¹³C-glucose breath test for diagnosing hepatic insulin resistance

To evaluate the hepatic insulin resistance with a simple and highly sensitive method, we developed the fasting ¹³C-glucose breath test (FGBT). The area under the curve until 6 hours (AUC₃₆₀) of the kinetic curve for ¹³C excretion was significantly lower in patients with impaired glucose tolerance than in healthy subjects. Also, the FGBT could be used to diagnose insulin resistance, with the homeostasis model assessment of insulin resistance as the gold standard, and to sensitively diagnose diabetes with fasting plasma glucose and HbA1c. We were able to set each cut-off value in the AUC₃₆₀. FGBT is a simple and highly sensitive and specific clinical test for evaluating hepatic carbohydrate metabolism.

(In collaboration with Meiji University, National Defense Medical College, and the Department of Surgery, The Jikei University)

2. New diagnostic marker transforming growth factor β latency-associated protein degradation products for diagnosis of fibrotic activity in the liver

We have developed a novel, quantitative, and specific assay of plasma latency-associated

protein (LAP) of transforming growth factor (TGF)- β degradation products (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. Also, immunostaining was performed for LAP-D in liver tissue sections, which were obtained at biopsy at the same time as blood sampling. Staining for LAP-D was positive in fibrotic bundles in cases of chronic active hepatitis C and cases of autoimmune hepatitis. For both these liver diseases LAP-D might be used as marker of the activity of liver fibrosis. (Supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation and performed in collaboration with the Institute of Physical and Chemical Research.)

3. Development of the human plasma protein high generating system using bioartificial liver

Plasma protein high-productive cells (functional cell line [FLC] 4) and FLC-4M#1 cells, which incorporates a strong promoter and a hybrid of the albumin gene, were cultured at high density in a 3-dimensional radial flow bioreactor to produce medical human albumin. In ASF-104 serum-free culture medium (Ajinomoto Pharmaceuticals Co. Ltd., Kawasaki), the quantity of albumin produced by FLC-4M#1 cells was approximately 3 times that produced by FLC-4 cells. However, in enhanced RDF serum-free culture medium (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo), the quantity of albumin produced by FLC-4 cells increased by a factor of 3. In contrast, the quantity of albumin produced by FLC-4 cells decreased by one-third. Because 40% to 50% of the protein released by FLC-4 cells is albumin, the quantity of albumin produced can be further increased through gene manipulation. The efficiency of the albumin secretion must be improved through posttranscriptional regulation.

(Supported by the Human Science Foundation and performed in collaboration with the National Institute for Infectious Diseases, Waseda University, and the Department of Biochemistry, The Jikei University)

4. Development of methods for the diagnosis and treatment of fatal encephalopathy with acute hepatic failure

A mouse model of fatal encephalopathy with acute hepatic failure was produced in the toxin receptor-mediated cell knockout (TRECK) hepatitis mouse. With magnetic resonance imaging we were able to confirm edema of the entire brain. Also, the plasma TGF- β LAP-D concentration was positively correlated with alanine aminotransferase activity and total bilirubin concentration and was negatively correlated with the total protein level. Immunohistochemical staining for LAP-D was correlated with that for α -smooth muscle cell actin in the TRECK hepatic failure model. This result suggests that the liver tissue of TRECK hepatitis mouse shows augmented fibrosis activity.

(Supported by Grant in Aid, Ministry of Education, Culture, Sports, Science and Technology and in collaboration with Advanced Institute of Science and Technology, Nara, and Medical Engineering Research Group, The Jikei University).

5. Development of an ultrasonic molecular imaging system

To develop an ultrasonic molecular imaging method, we performed a study of the application of high-sensitivity detection technology using micro-nano bubbles that were highly stable and provided a high degree of contrast with high-level harmonics. The stability of microbubbles in the serum was increased by a mixture of phospholipids and a newly developed detergent.

(Performed in collaboration with the Tokyo University of Science and the Departments of Radiology and Biochemistry, The Jikei University).

Clinical psychiatry

Psychotropic drugs give rise to some concerns in clinical practice because of their ability to reduce seizure threshold; therefore, we examined the safety and efficacy of psychotropic drugs in several forms of psychosis associated with epilepsy. We reported a case of epilepsy in a patient with ring chromosome 20 syndrome (R 20 synd), and from a review of the literature, we discussed the characteristics of patients with R 20 synd. Furthermore, we reported a case of adult epilepsy and ictal apnea during sleep. A study was performed to prevent the recurrence of depression in patients with epilepsy.

Publications

Fujise K, Tatsuzawa K, Kono M, Hoshina S, Tsubota A, Niiya M, Namiki Y, Tada N, Tajiri H. A mutation of the start codon in the X region of hepatitis B virusDNA in a patient with non-B, non-C chronic hepatitis. *World J Hepatol* 2011; **3**: 56-60.

Saito R, Ishii Y, Ito R, Nagatsuma K, Tanaka K, Saito M, Maehashi H, Nomoto H, Ohkawa K, Mano H (Josai Univ), Aizawa M (Meiji Univ), Hano H, Yanaga K, Matsuura T. Transplantation of liver organoids in the omentum and kidney. Artif Organs 2011; 35: 80-3.

Suzuki M, Ishiyama I. Reference interval of maximal oxygen uptake (VO2max) as one of the determinants of health-related physical fitness in Japan. *Tairyoku Kagaku* 2010; **59:** 75-85.

Hirai N, Horiguchi S, Ohta M, Watanabe M, Shioji I, Ohnishi A. Elevated urinary biopyrin excretion and oxidative bilirubin metabolism during 24-hour ultramarathon running. *Rinsyo Byori* 2010; **58:** 313-8.

Fukuda M, Kawahara Y, Hirota T, Akizuki S, Murakami S, Nakajima H, leiri I, Ohnishi A. Genetic polymorphisms of hepatic ABC-transporter in patients with heoatocellular carcinoma. *J Cancer Ther* 2010; **1**: 114-23.

Matsuo S, Yamane T, Date T, Hioki M, Ito K, Narui R, Tanigawa S, Nakane T, Hama Y, Tokuda M, Yamashita S, Aramaki Y, Inada K, Shibayama K, Miyanaga S, Yoshida H, Miyazaki H, Abe K, Sugimoto K, Taniguchi I, Yoshimura M. Comparison of the clinical outcome after pulmonary vein isolation based on the appearance of adenosine-induced dormant pulmonary vein conduction. *Am Heart J* 2010; **160**: 337-45.

Matsuo S, Yamane T, Date T, Lellouche N, Tokutake K, Hioki M, Ito K, Narui R, Tanigawa S, Nakane T, Tokuda M, Yamashita S, Aramaki Y, Inada K, Shibayama K, Miyanaga S, Yoshida H, Miyazaki H, Abe K, Sugimoto K, Taniguchi I, Yoshimura M. Dormant pulmonary vein conduction induced by adenosine in patients with atrial fibrillation who underwent catheter ablation. Am Heart J 2011; **161:** 188-96.

Yoshida H, Yanai H, Ito K, Tomono Y, Koikeda T, Tsukahara H, Tada N. Administration of natural astaxanthin increases serum HDL-cholesterol and adiponectin in subjects with mild hyperlipidemia. *Atherosclerosis* 2010; **209:** 520-3.

Hirowatari Y, Yoshida H, Kurosawa H, Shimura Y, Yanai H, Tada N. Analysis of cholesterol levels in lipoprotein(a) with anion-exchange chromatography. J Lipid Res 2010; 51: 1237-43. Yoshida H, Shimizu M, Ikewaki K, Taniguchi I, Tada N, Yoshimura M, Rosano G, Dahlöf B, Mochizuki S; Jikei Heart Study Group. Sex differences in effects of valsartan administration on cardiovascular outcomes in hypertensive patients: findings from the Jikei Heart Study. J Hypertens 2010; 28: 1150-7.

Miyamoto Y, Onoue K, Nishioka M, Nakata N, Matsuura T, Asakura T, Ohkawa K, Tsuchiya K¹, Itani K², Konno T², Sakai H¹, Abe M¹ (¹Tokyo Univ Sci, ²Aloka Co., Ltd). Experimental study of the stability of sonazoid[™]. Jikeikai Med J 2010; 57: 55-60.

Reviews and Books

Yoshida H, Kisugi R. Mechanisms of LDL oxidation. *Clin Chim Acta* 2010; **411:** 1875-82.

Yoshida H. Broadly-defined apolipoproteins identified by proteomic analysis (in Japanese). *Rinsho Kensa* 2010; **54:** 359-67.

Yoshida H. Front line of oxidized lipoproteins: Role of oxidized lipoproteins in atherogenesis and cardiovascular disease risk (in Japanese). *Rinsho Byori* 2010; **58**: 622-30.

Yoshida H. Clinical significance of lipoprotein

analysis method by HPLC (in Japanese). *Rinsho Byori* 2010; **58:** 1093-8.

Department of Internal Medicine Division of Gastroenterology and Hepatology

Hisao Tajiri, Professor Mikio Zeniya, Professor Ichiro Takagi, Professor Hirokazu Nishino, Professor Tomohisa Ishikawa, Associate Professor Mika Matsuoka, Assistant Professor Satoshi Suto, Assistant Professor Mario Itsubo, Professor Toshifumi Okusa, Professor Yoshio Aizawa, Professor Shigeo Koido, Associate Professor Atsushi Hokari, Associate Professor Kazuhiko Koike, Assistant Professor Yoshinari Miyagawa, Assistant Professor

Research Activities

Alimentary tract

We analyzed the efficacy of polaprezinc enema in the treatment of ulcerative colitis. Endoscopic and clinical findings showed that the addition of polaprezinc enemas produced significant improvement in patients with moderate to severe ulcerative colitis. Significant improvements were detected with endoscopy in the rectum and sigmoid colon, which are the areas exposed to polaprezinc enemas. A polaprezinc enema may become a useful new add-on treatment to accelerate mucosal healing in ulcerative colitis.

In a double-blind placebo-controlled multicenter trial, 2-week triple antibiotic therapy produced improvement, remission, and steroid withdrawal in active ulcerative colitis more effectively than did a placebo.

The efficacy and safety of endoscopic balloon dilatation for the treatment of intestinal strictures were studied. A single-balloon enteroscopy system was used to perform 222 enteroscopic procedures.

The subjects who underwent endoscopic balloon dilation were 12 patients with Crohn's disease and 1 patient with a nonspecific ulcer of the small intestine. The procedures allowed surgery to be avoided in 10 patients (76.9%). Complications developed in only 1 of the 13 patients (7.7%). Endoscopic balloon dilatation appears to be safe and extremely useful, allows surgical resection to be avoided, and maintains patients' quality of life.

Statistical analysis showed that the strongest risk factor for metastasis of superficial carcinoma of the esophagus to the lymph nodes is vascular invasion, as evaluated with a special staining procedure.

Intestinal Behçet's disease is rare. The diagnosis and management of intestinal Behçet's disease is sometimes difficult and controversial. We reported 5 cases, including a case complicated by myelodysplastic syndrome, and discussed therapeutic strategies.

Liver

Clinicopathological study of autoimmune hepatitis: The prediction of resistance to immunosuppressive therapy and of recurrence despite immunosuppressive therapy were weighed against pathological findings of continuance in liver biopsies specimens in cases of autoimmune hepatitis (AIH). Intrahepatic natural killer T-cell kinetics in AIH model mice: The profiles of natural killer (NK) T-cells and several cytokines were examined in AIH model mice. Changes in immunoreactions were analyzed in knock-out model mice.

Treatment response of antiviral analogue nucleic acids in chronic hepatitis B infection: Analyses of the viral gene sequence and treatment response were performed. We considered the possibility of a new concurrent therapy for chronic hepatitis B.

Nutritional evaluation in nonalcoholic fatty liver disease: The nutritional background in nonalcoholic fatty liver disease may be evaluated by using a food frequency questionnaire based on food groups and indirect calorimetry.

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

Study of hepatitis C virus: The initial immune response to the hepatitis C virus (HCV) was studied in transgenic mice. We found that NK cells participate in the elimination of core-expressing hepatocytes in the innate immune response during the acute phase of HCV infection.

In a study with HCV transgenic mice, the HCV core protein stimulated immune response to lymphocytes at liver parenchyma. NK cells participate in the elimination of core-expressing hepatocytes in the innate immune response during the acute phase of HCV infection.

Multiregression analysis revealed that serum levels of apolipoprotein B and amino-acid substitution at position 70 of the HCV core region are independent factors associated with the efficacy of pegylated interferon plus ribavirin therapy for chronic HCV G1b infection. In addition, the usefulness of this response-guided therapy on chronic hepatitis C was verified in our cohort.

Magnetic resonance imaging assessment of hepatocellular carcinoma: The magnetic resonance imaging properties with contrast enhancement were related to prognosis and clinical characteristics.

Examination of the molecular mechanisms of the differentiation of hepatic stem cells: Transcription of the marker factor Sall4 regulated cell-fate decisions in fetal hepatic stem/ progenitor cells. This molecule has the potential for clinical application.

The fasting ¹³C-glucose breath test: The area under the curve until 360 minutes (AUC₃₆₀) of the kinetic curve for ¹³C excretion of the fasting ¹³C-glucose breath test (FGBT) reflects the efficiency of energy production in the liver. The AUC₃₆₀ of healthy subjects was consistently higher than that of patients. FGBT is a novel glucose metabolic test that can be used to conveniently and safely evaluate the balance of glucose metabolism in the liver. The FGBT test has excellent sensitivity for diagnosing alterations in hepatic glucose metabolism.

Latency-associated protein of tumor necrosis factor β degradation products: We have developed a novel quantitative and specific assay of plasma latency-associated proteins (LAPs), which are produced during proteolytic activation of tumor necrosis factor (TNF) β . The LAP degradation products would be novel markers in blood and tissues reflecting fibrogenetic activity but not the amount of accumulated fibrosis in patients, especially those with chronic HCV or AIH, and can be used to estimate the early stage of fibrosis,

when TNF- β activation and hepatic stellate cell activation are more frequent, and to assess the effects of treatment.

Pancreas

Gemcitabine enhances Wilms' tumor gene WT1 expression and sensitizes human pancreatic cancer cells with WT1-specific T-cell-mediated antitumor immune responses.

Fusions of dendritic cells from healthy donors and plasma protein fraction-dependent pancreatic cancer cells were effective for inducing antitumor immunity. However, fusions from patients with metastatic pancreatic cancer induced increased expression levels of TGF- β 1 in CD4+ CD25-high T cells and induced low levels of CTLs of cytotoxic T lymphocytes with decreased interferon γ production.

Publications

Yamane T, Uchiyama K, Ishii T, Nakano M, Kanetuna Y, Okusa T, Tajiri H. Isolated granulomatous gastritis showing discoloration of lesions after Helicobacter pylori eradication. *Dig Endosc* 2010; **22:** 140-3.

Koido S, Hara E, Homma S, Namiki Y, Komita H, Takahara A, Nagasaki E, Ito M, Sagawa Y, Mitsunaga M, Uchiyama K, Sato K, Arihiro S, Ohkusa T, Gong J, Tajiri H. Dendritic/pancreatic carcinoma fusions for clinical use: comparative functional analysis of healthy-versus patientderived fusions. *Clin Immunol* 2010; **135**: 384-400.

Yoshimura N, Goda K, Tajiri H, Ikegami M, Nakayoshi T, Kaise M. Endoscopic features of nonampullary duodenal tumors with narrow-band imaging. *Hepatogastroenterology* 2010; **57**: 462-7.

Kaise M, Kato M, Tajiri H. High-definition endoscopy and magnifying endoscopy combined with narrow band imaging in gastric cancer. *Gastroenterol Clin North Am* 2010; **39:** 771-84.

Imazu H, Uchiyama Y, Matsunaga K, Ikeda K, Kakutani H, Sasaki Y, Sumiyama K, Ang TL, Omar S, Tajiri H. Contrast-enhanced harmonic EUS with novel ultrasonographic contrast (Sonazoid) in the preperative T-staging for pancreaticobiliary malignancies. Scand J Gastroenterol 2010; **45:** 732-8.

Aizawa M, Tsubota A, Fujise K, Sato K, Baba M, Takamatu M, Namiki Y, Ohkusa T, Tajiri H. Overlap/switch to adefovir monotherapy for lamivudine-resistant patients who responded to combination therapy: a pilot controlled study. *Intern Med* 2010; **49**: 1067-72.

Sumiyama K, Tajiri H, Gostout CJ, Kawamura M, Imazu H, Ohya TR, Ikeda K, Goda K, Saito S, Kato T. Chemically assisted submucosal injection facilitates endoscopic submucosal dissection of gastric neoplasms. *Endoscopy* 2010; **42**: 627-32.

Kato M, Kaise M, Yonezawa J, Toyoizumi H, Yoshimura N, Yoshida Y, Kawamura M, Tajiri **H.** Magnifying endoscopy with narrow-band imaging achieves superior accuracy in the differential diagnosis of superficial gastric lesions identified with white-light endoscopy: a prospective study. *Gastrointest Endosc* 2010; **72**: 523-9.

Kaneyama H, Kaise M, Arakawa H, Arai Y, Kanazawa K, Tajiri H. Gastroesophageal flap valve status distinguishes clinical phenotypes of large hiatal hernia. World J Gastroenterol 2010; 16: 6010-5.

Sumiyama K, Utsunomiya K, Ohya T, Aihara H, Ikeda K, Imazu H, Tamai N, Nagano H, Ishinoda Y, Tajiri H. A pilot study on ultrasoundassisted liposuction of the greater omentum in porcine models. *Minim Invasive Ther Allied Technol Epub* 2011 Mar 14.

Ikeda K, Sumiyama K, Tajiri H, Yasuda K, Kitano S. Evaluation of a new multitasking platform for endoscopic full-thickness resection. *Gastrointest Endosc* 2011; **73**: 117-22.

Fujise K, Tatsuzawa K, Kono M, Hoshina S, Tsubota A, Niiya M, Namiki Y, Tada N, Tajiri H. A mutation of the start codon in the X region of hepatitis B virus DNA in a patient with non-B, non-C chronic hepatitis. *World J Hepatol* 2011; **3**: 56-60.

Rey JF, Ogata H, Hosoe N, Ohtuka K, Ogata N, Ikeda K, Aihara H, Pangtay I, Hibi T, Kudo S, Tajiri H. Feasibility of stomach exploration with a guided capsule endoscope. *Endoscopy* 2010; 42: 541-5.

Tamai N, Matsuda K, Sumiyama K, Isshi K, Narimiya N, Tajiri H. Prophylactic hemostasis for postpolypectomy mucosal defect using endoclip under infrared imaging endoscopy. *Surg Technol Int* 2010; **19**: 91-6.

Nagasaki E, Takahara A, Koido S, Sagawa Y, Aiba K, Tajiri H., Yagita H, Homma S. Combined treatment with dendritic cells and 5-fluorouracil elicits augmented NK cell-mediated antitumor activity through the tumor necrosis factoralpha pathway. J Immunother 2010; **33:** 467-74.

Department of Internal Medicine Division of Neurology

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

General Summary

Our research in 2010 included the following: 1) an actigraphic study of tremor treated with zonisamide in patients with Parkinson's disease (PD), 2) a study of autonomic dysfunction in neurodegenerative disease, 3) neurophysiological studies of the visual information processing functions in neurodegenerative disease and of diabetic polyneuropathy, 4) neuroradiological studies with nuclear medicine, 5) ultrasonographic studies of cerebrovascular disease, and 6) basic research on motor neuron disease and axonal plasticity of the central nervous system.

Research Activities

Actigraphic study of tremor before and after treatment with zonisamide in patients with PD

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

Autonomic dysfunction in neurodegenerative disease

We studied the characteristics of subclinical autonomic nervous dysfunction in *de novo* PD without orthostatic hypotension. Autonomic nervous function, including cardiac sympathetic gain, was evaluated on the basis of cardiac radioiodinated metaiodobenzyl-guanidine uptake, the response to the Valsalva maneuver, and spectral analyses of the RR interval and systolic blood pressure in 20 patients who had de novo PD without orthostatic hypotension. Decreased cardiac metaiodobenzylguanidine uptake was found even in patients who had PD without orthostatic hypotension. We also observed a reduced response to the Valsalva maneuver, and performed spectral analyses of the RR interval and systolic blood pressure. These results show that latent cardiac and vasomotor sympathetic dysfunction, but not parasympathetic dysfunction, is already present in early *de novo* PD, even without orthostatic hypotension. We also studied the relation of olfactory

dysfunction to cardiovascular dysautonomia in patients with PD. We found that olfactory dysfunction in PD was significantly related to both cardiac sympathetic and parasympathetic dysfunction, as well as vascular sympathetic dysfunction. As nonmotor symptoms of PD, olfactory dysfunction and autonomic network failure appear to be closely related in PD.

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

Olfactory dysfunction in Alzheimer's disease (AD) was also evaluated with a simple method using an incense stick. The subjects were 93 healthy control subjects and 16 patients with AD. The rate of olfactory dysfunction was significantly higher in patients with AD than in healthy control subjects. Furthermore, olfactory dysfunction in AD was correlated with scores on the Hasegawa Simple Intelligence Scale (revised edition).

We investigated the volume of the olfactory bulb in PD and PD-related diseases, including multiple system atrophy, progressive supranuclear palsy, corticobasal degeneration, and other neurodegenerative diseases. Olfactory bulb volume was less in patients with PD than in patients with PD-related diseases. These results are compatible with those of our postmortem study of the olfactory bulb. Magnetic resonance may be useful in the differential diagnosis of PD and PD-related diseases. We plan to perform further examinations.

Neurophysiological studies of the visual information processing functions in neurodegenerative disease and of diabetic polyneuropathy

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

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

Neuroradiological studies with nuclear medicine

Neuroradiological studies were applied to neurodegenerative disorders, including dementia and parkinsonism. Brain perfusion images were compared by means of statistical imaging methods, such as 3-dimensional stereotactic surface projection analysis of iodoamphetamine single-photon emission computed tomography (SPECT) and easy Z-score imaging system analysis of 99mTc-ethyl cysteinate dimer SPECT among patients with dementia and parkinsonian disorders. These novel methods demonstrated the spectrum of pathological involvement of cholinergic and dopaminergic projections in AD and PD and may be useful in routine clinical practice.

Ultrasonographic studies of cerebrovascular disease

Cerebrovascular ultrasonography was useful for rapidly evaluating cerebral hemodynamics in real time for patients with acute ischemic stroke. We evaluated the occlusion of intracranial arteries with transcranial color flow imaging and monitored residual flow in real time every 15 minutes for 120 minutes after bolus infusion of tissue plasminogen activator (t-PA). We were able to monitor residual flow in 4 patients who had good echo windows. Two patients had very early complete recanalization within 60 minutes after bolus infusion of the t-PA; however, occlusion persisted during the 120 minutes of monitoring in a patient with proximal occlusion of the middle cerebral artery. The National Institutes of Health Stroke Scale of 2 patients with very early recanalization was 0 at the end of treatment. Symptomatic or asymptomatic intracranial hemorrhage occurred only in patients without recanalization. Real-time ultrasound monitoring is useful for evaluating the very early thrombolytic effects of t-PA associated with early clinical recovery.

Clarifying the mechanism underlying the selective vulnerability of motoneurons

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

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 for promoting axonal reorganization or functional recovery after cortical injury.

Publications

A(2A) receptors measured with[C]TMSX PET in the striata of Parkinson's disease patients. *PLoS One* 2011; **6**: e17338.

Omoto S, Ueno M¹, Mochio S, Takai T², Yamashita T¹ ('Osaka Univ, ²Tohoku Univ). Genetic deletion of paired immunoglobulin-like receptor B does not promote axonal plasticity or functional recovery after traumatic brain injury. J

Oka H, Toyoda C, Yogo M, Mochio S. Olfactory dysfunction and cardiovascular dysautonomia in Parkinson's disease. *J Neurol* 2010; **257**: 969-76.

Mishina M, Ishiwata K, Naganawa M, Kimura Y, Kitamura S, Suzuki M, Hashimoto M, Ishibashi K, Oda K, Sakata M, Hamamoto M, Kobayashi S, Katayama Y, Ishii K. Adenosine

Neurosci 2010; 30: 13045-52.

Omoto S, Ueno M¹, Mochio S, Yamashita T¹ (**'Osaka Univ).** Corticospinal tract fibers cross the ephrin-B³-negative part of the midline of the spinal cord after brain injury. *Neurosci Res* 2011; **69:** 187-95.

Umehara T, Yaguchi H, Suzuki M, Isozaki E, Mochio S. Are hypersegmented neutrophils a characteristic of Boucher-Neuhäuser syndrome? *J Neurol Sci* 2010; **295:** 128-30.

Reviews and Books

Kurita A. Event-related otential analyses of brain functions in Parkinson's disease (in Japanese). *Nihon Yakubutsu Noha Gakkai Zasshi* 2010; **11**: 45-52.

Kurita A. Recent advances in visual eventrelated potential studies (in Japanese). *Rinsho Shinkei Seirigaku* 2010; **38**: 148-53.

Department of Internal Medicine Division of Kidney and Hypertension

Tatsuo Hosoya, Professor and Chairperson Tetsuya Kawamura, Associate Professor Keitaro Yokoyama, Assistant Professor Kazushige Hanaoka, Assistant Professor Yoichi Miyazaki, Assistant Professor Takashi Yokoo, Assistant Professor Iwao Ohno, Professor Yasunori Utsunomiya, Associate Professor Makoto Ogura, Assistant Professor Masato Ikeda, Assistant Professor Hiroshi Hayakawa, Assistant Professor

General Summary

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

Research Activities

Nephrology

1. Glomerulonephritis

Recently, steroid pulse therapy has been recommended as a treatment for patients with progressive immunoglobulin A nephropathy. We demonstrated that steroid pulse therapy significantly decreases the severity of proteinuria. In addition, tonsillectomy, in combination with steroid pulse therapy, can induce clinical remission in patients with immunoglobulin A nephropathy.

We generated and analyzed inducible and podocyte-specific vascular endothelial growth factor transgenic mice using the Tet-On system. Our results indicate that dysregulation of vascular endothelial growth factor expression leads to the alteration of glomerular capillary formation via dysfunction of both endothelial and mesangial cells.

Metanephros transplantation significantly reduced vascular Ca and Pi content. Von Kossa staining of the media of the thoracic aorta indicated that metanephros transplantation suppresses the progression of vascular calcification.

2. Dialysis and kidney transplantation

We evaluated the clinical value of combined therapy with peritoneal dialysis and hemodialysis and found that it is a useful way of controlling body fluids and that peritoneal function can be maintained over a long period of treatment time (*Clinical Nephrology*, 2010). Moreover, we evaluated peritoneal histology. In addition, we studied vascular calcification in patients with end-stage renal disease (*Clinical Nephrology*, 2010).

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 (*American Journal of Transplantation*, 2008). We found an association between peritubular capillary endothelial c-Jun activation and interstitial fibrosis in chronic antibody-mediated rejection.

Hypertension

A study of awareness of home blood pressure (BP) measurement among practitioners who deal with hypertension was a main research project in 2010. This study was unique in that it focused specifically on home BP measurement. Because the guidelines released by the Japanese Society of Hypertension in 2008 are the only guidelines to include home-based BP, we believe highlighting the importance of home-based BP measurement is important. The study has been completed and was submitted to the journal *Blood Pressure*.

In patients with chronic kidney disease, T-type calcium channels can be overexpressed in the kidney. In addition, blocking of T-type calcium channels leads to inhibition of Rhokinase activity and renal interstitial. We extended this result to further investigate the cardiorenal continuum by introducing 5/6 nephrectomized rats. We are now analyzing laboratory and histological findings.

Uric acid metabolism

We investigated the hypouricemic effect of a novel synthesized xanthine oxidase inhibitor, topiroxostat, in patients who have hyperuricemia with and without gout. Topiroxostat showed dose responsiveness in its ability to lower serum levels of uric acid, and doses of both 80 mg and 120 mg produced significantly greater increases in estimated glomerular filtration rate than did placebo.

Publications

Gheisari Y, Yokoo T, Matsumoto K, Fukui A, Sugimoto N, Ohashi T, Kawamura T, Hosoya T, Kobayashi E. A thermoreversible polymer mediates controlled release of GDNF to enhance kidney regeneration. *Artif Organs* 2010; **34:** 642– 7.

Terawaki H, Takada Y¹, Era S¹, Funakoshi Y², Nakayama K³, Nakayama M³, Ogura M, Ito S³, Hosoya T (¹Gifu Univ, ²Funakoshi Clin, ³Tohoku Univ). The redox state of albumin and serious cardiovascular incidence in hemodialysis patients. Ther Apher Dial 2010; **14:** 465-71.

Terawaki H, Yokoyama K, Yamada Y, Maruyama Y, Iida R, Hanaoka K, Yamamoto H, Obata T, Hosoya T. Low-grade endotoxemia is contributing to chronic inflammation in hemodialysis patients: examination with the novel lipopolysaccharide detection method. *Ther Apher Dial* 2010; **14:** 477-82.

Otani H¹, Kikuya M¹, Hara A¹, Terata S¹, Ohkubo T¹, Kondo T¹, Hirose T¹, Obara T¹, Metoki H¹, Inoue R¹, Asayama K¹, Kanno A¹, Terawaki H, Nakayama M¹, Totsune K¹, Hoshi H¹, Satoh H¹, Izumi S¹, Imai Y¹ (¹Tohoku Univ). Association of kidney dysfunction with silent lacunar infarcts and white matter hyperintensity in the general population: the Ohasama study. Cerebrovasc Dis 2010; **30:** 43–50.

Terawaki H, Nakao M, Nakayama K¹, Nakayama M¹, Kimura A, Takane K, Mitome J, Hamaguchi A, Ogura M, Yokoyama K, Ito S, Hosoya T (¹Tohoku Univ). Peritoneal clearance and transport of methylglyoxal. Nephrol Dial Transplant 2011; **26:** 754-5.

Okonogi H, Utsunomiya Y, Miyazaki Y, Koike K, Hirano K, Tsuboi N, Suzuki T, Hara Y, Ogura M, Hosoya T, Kawamura T. A predictive clinical grading system for immunoglobulin a nephropathy by combining proteinuria and estimated glomerular filtration rate. *Nephron Clin Pract* 2011; **118**: c292-300.

Yokoyama K, Urashima M, Ohkido I, Kono T, Yoshida T, Muramatsu M, Niu T, Hosoya T. L-type voltage-dependent calcium channel alpha subunit 1C is a novel candidate gene associated with secondary hyperparathyroidism: an application of haplotype-based analysis for multiple linked single nucleotide polymorphisms. Nephron Clin Pract 2010; **115:** c237-43.

Utsunomiya Y, Hara Y, ItoH, Okonogi H, Miyazaki Y, Hashimoto Y (Univ Toyama), Hosoya T. Effects of probenecid on the pharmacokinetics of mizoribine and co-administration of the two drugs in patients with nephrotic syndrome. Int J Clin Pharmacol Ther 2010; **48:** 751-5.

Matsuo N, Yokoyama K, Maruyama Y, Ueda Y, Yoshida H, Tanno Y, Yamamoto R, Terawaki H, Ikeda M, Hanaoka K, Yamamoto H, Ogura M, Watanabe S, Kimura Y, Hosoya T. Clinical impact of a combined therapy of peritoneal dialysis and hemodialysis. *Clin Nephrol* 2010; **74:** 209-16.

Ohkido I, Yokoyama K, Kagami S, Hosoya T. The hypothesis that bone turnover influences FGF23 secretion. *Kidney Int* 2010; **77**: 743.

Yoshida H, Yokoyama K, Yaginuma T, Ohkido I, Yamamoto H, Utsunomiya Y, Kawakami M (Seirei Sukura Citizen Hosp), Hosoya T. Difference in coronary artery intima and media calcification in autopsied patients with chronic kidney disease. *Clin Nephrol* 2011; **75:** 1-7.

Mitome J, Yamamoto H, Maruyama Y, Kobayashi A, Yaginuma T, Matsuo N, Tanno Y, Hayakawa H, Miyazaki Y, Yokoyama K, Utsunomiya Y, Yamaguchi Y, Hosoya T. Successful treatment of recurrent focal segmental glomerulosclerosis combined with calcineurin inhibitor nephrotoxicity four yr after kidney transplantation. *Clin Transplant* 2010; **24 Suppl 22:** 48-53.

Kanno A¹, Metoki H¹, Kikuya M¹, Terawaki H,

Hara A¹, Hashimoto T¹, Asayama K¹, Inoue R¹, Shishido Y¹, Nakayama M¹, Totsune K¹, Ohkubo T¹, Imai Y¹ (¹Tohoku Univ). Usefulness of assessing masked and white-coat hypertension by ambulatory blood pressure monitoring for determining prevalent risk of chronic kidney disease: the Ohasama study. *Hypertens Res* 2010; **33**: 1192-8.

Reviews and Books

Terawaki H, Era S (Gifu Univ), Nakayama M, Hosoya T. Decrease in reduced-form albumin among chronic kidney disease patients: new insights in cardiovascular complications. Ther Apher Dial 2011; 15: 156-60. Epub 2011 Jan 25. Yokoo T, Yanagita M. Stem cell therapy against oxidative stress and hypoxia. In: Miyata T, Eckardt KU, Nangaku M, editors. Studies in renal disorders. New York: Springer; 2011. p. 673-88.

Department of Internal Medicine Division of Rheumatology

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

General Summary

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

Research Activities

Clinical and experimental studies of autoimmune disease were performed.

1. Analysis of the relationship of neovascularization in autoimmune animal models Several studies have found that neovascularization inhibitors inhibit the development of arthritis in animal models of rheumatoid arthritis. We evaluated the effects of the neovascularization inhibitor endostatin in murine models of collagen-induced arthritis and bleomycin-induced pulmonary fibrosis.

Furthermore, we are investigating whether there was any relation between *Bombina variegata* peptide 8 and angiogenesis in autoimmune arthritis.

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

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

3. Histopathological comparison between dermatomyositis and polymyositis

We have obtained specimens of muscle, fascia, and skin through "en-bloc biopsy" from patients with dermatomyositis or polymyositis under the guidance of magnetic resonance imaging. We then histopathologically investigated the severity of inflammation around the muscle, fascia, and subcutaneous tissue to determine differences between dermatomyositis and polymyositis.

4. Clinical studies aimed at standardizing immunosuppressant therapy for autoimmune diseases

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

Publications

Kurosaka D, Hirai K, Nishioka M, Miyamoto Y, Yoshida K, Noda K, Ukichi T, Yanagimachi M, Furuya K, Takahashi E, Kingetsu I, Fukuda K, Yamada A. Clinical significance of serum levels of vascular endothelial growth factor, angiopoietin-1, and angiopoietin-2 in patients with rheumatoid arthritis. J Rheumatol 2010; **37**: 1121-8. Yoshida K, Kurosaka D, Joh K, Matsushima S, Takahashi E, Hirai K, Noda K, Ukichi T, Furuya K, Yanagimachi M, Kingetsu I, Fukuda K, Yamada A. Fasciitis as a common lesion of dermatomyositis, demonstrated early after disease onset by en bloc biopsy combined with magnetic resonance imaging. *Arthritis Rheum* 2010; **62**: 3751–9.

Department of Internal Medicine Division of Cardiology

Michihiro Yoshimura, Professor Ikuo Taniguchi, Professor Teiichi Yamane, Associate Professor Takahiro Shibata, Assistant Professor Atsushi Seo, Assistant Professor Hidenori Yagi, Assistant Professor Takayuki Ogawa, Assistant Professor Mitsuyuki Shimizu, Professor Shingo Seki, Associate Professor Kenichi Hongo, Associate Professor Naofumi Aoyama, Assistant Professor Makoto Kawai, Assistant Professor Kimiaki Komukai, Assistant Professor Toshio Hasuda, Assistant Professor

General Summary and Research Activities

Research in every field, both clinical and basic, is being driven by the desire to obtain reliable results.

Clinical research

In clinical research, we have been participating in multicenter collaborative studies, including large-scale clinical studies, and conducting research during routine clinical practice. In the large-scale clinical studies, we have primarily collaborated in subanalyses in the Japanese Investigation of Kinetic Evaluation in Hypertensive Event And Remodeling Treatment (JIKEI HEART) Study, whose results were reported last year, and in such studies as the Japanese Rhythm Management Trial for Atrial Fibrillation II (J-RHYTHM II) (a multicenter study comparing upstream drug therapy with calcium antagonists and angiotensin receptor blockers for atrial fibrillation associated with hypertension), the Assessment of β -Blocker Treatment in Japan Chronic Heart Failure (J-CHF) study (a large-scale clinical study to establish a β -blocker treatment methods in chronic heart failure), the Pitavastatin Heart Failure (PEARL) study (a multicenter cooperative study to investigate the ameliorative effects of hydroxymethylglutaryl 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 tomographic examinations of the coronary arteries.

We have converted patient data, including risk factors and lesion morphology, during catheter examinations and treatment in various clinical research divisions into a database and have performed a study comparing risk factors, outcome, and other aspects of ischemic heart disease, cardiomyopathy, and other conditions. In addition, we have participated in nationwide clinical studies, mainly detailed investigations of treatment with drug-eluting stents and the diagnosis of coronary vasospasm, which is closely related to the etiology of ischemic heart disease. These studies include: Japan-Drug Eluting Stents Evaluation; a Randomized Trial (J-DESSERT), Coronary Spasm Association (CSA), Japan Unprotected Left Main Coronary Artery Disease Percutaneous Coronary Intervention Strategy On New Generation Stents (J-LESSON), and Optimal Duration of DAPT

Following Treatment With Endeavor (Zotarolimus-eluting Stent) in Real-world Japanese Patients (OPERA).

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

We have been aggressively treating atrial fibrillation by catheter ablation, and during this fiscal year we have treated approximately 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 basic science and clinical science graduate students and presentation of the results of many studies. In the field of arrhythmia, we have used various experimental models to study the development of atrial fibrillation in regard to the effects on the myocardium of inflammatory cell invasion mechanisms and fibrosis. In the field of cardiomyocyte physiology, we have investigated the physiological and pathophysiological regulatory mechanisms of myocardial contraction and relaxation and performed a study using molecular biology techniques and physiological techniques. We have also studied 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 in which dilated cardiomyopathy develops as a result of troponin T mutations. In the field of myocardial metabolism, we have investigated the association between ischemia-reperfusion damage and intracellular ion kinetics in isolated perfused hearts of type-2 diabetic mice.

Publications

Yagi H, Komukai K, Hashimoto K, Kawai M, Ogawa T, Anzawa R, Minai K, Nagoshi T, Ogawa K, Taniguchi I, Yoshimura M. Difference in risk factors between acute coronary syndrome and stable angina pectoris in the Japanese: Smoking as a crucial risk factor of acute coronary syndrome. J Cardiol 2010; **55**: 345-53.

Morikawa Y¹, Mizuno Y¹, Harada E¹, Kuboyama O¹, Yoshimura M, Yasue H¹ ('Kumamoto Aging Res Inst). Nitrate tolerance as a possible cause of multidrug-resistant coronary artery spasm. Int Heart J 2010; **51**: 211-3.

Yoshimura M, Kawai M. Synergistic inhibitory effect of angiotensin II receptor blocker and thiazide diuretic on the tissue renin-angiotensin-aldosterone system. *J Renin Angiotensin Aldosterone Syst* 2010; **11**: 124–6. Matsuo S, Yamane T, Hioki M, Narui R, Ito K, Yamashita S, Tokuda M, Yoshida H, Date T, Sugimoto K, Yoshimura M. Acute progression of congestive heart failure during paroxysmal supraventricular tachycardia in a patient without structural heart disease. J Cardiol Cases 2010; 1: e133-6.

Matsuo S, Yamane T, Date T, Yoshimura M. Spontaneously isolated sinus node activation in sick sinus syndrome as revealed by a three-dimensional mapping system. *Heart Rhythm* 2010; **7:** 856-7.

Yoshida H, Shimizu M, Ikewaki K, Taniguchi I, Tada N, Yoshimura M, Rosano G, Dahlof B, Mochizuki S; Jikei Heart Study group. Sex differences in effects of valsartan administration on cardiovascular outcomes in hypertensive patients: findings from the Jikei Heart Study. *J Hypertens* 2010; **28:** 1150-7.

Komukai K, O-Uchi J, Morimoto S, Kawai M, Hongo K, Yoshimura M, Kurihara S. Role of Ca2+/calmodulin-dependent protein kinase II in the regulation of the cardiac L-type Ca2+ current during endothelin-1 stimulation. *Am J Physiol Heart Circ Physiol* 2010; **298:** H1902-7.

Matsuo S, Yamane T, Date T, Hioki M, Narui R, Ito K, Yamashita S, Tokuda M, Yoshida H, Yoshimura M. A pause in pulmonary vein activity during atrial fibrillation: What is the mechanism ? Pacing Clin Electrophysiol 2010; **33**: 882-4.

Matsukage T¹, Masutani M², Yoshimachi F³, Takahashi A⁴, Katsuki T⁵, Saito S⁶, Terai H⁷, Katahira Y⁶, Uehara Y, Tohara S⁹, Ohba Y¹⁰, Shinohara S¹¹, Asano H¹², Matsumura T¹³, Hata T¹⁴, Ikari Y¹; PIKACHU Registry Investigators ('Tokai Univ Sch Med, ²Hyogo Coll Med, ³Aomori Pref Ctrl Hosp, ⁴Takahashi Hosp, ⁵Jichi Med Univ, ⁶Shonan Kamakura Gen Hosp, ¹Kanazawa Cardiovasc Hosp, ⁸Tohoku Kouseinenkin Hosp, ⁹Kasukabe Chuo Gen Hosp, ¹⁰Chihaya Hosp, ¹¹Takai Hosp, ¹²Tosei Gen Hosp, ¹³Kumamoto Rosai Hosp, ¹⁴Kyoto Kujo Hosp). A prospective multicenter registry of 0.010-inch guidewire and compatible system for chronic total occlusion: The PIKACHU registry. *Catheter Cardiovasc Interv* 2010; **75**: 1006-12.

Toko H¹, Takahashi H¹, Kayama Y, Okada S¹, Minamino T¹, Terasaki F², Kitaura Y², Komuro I^{1.3} (¹Chiba Univ Grad Sch Med, ²Osaka Med Coll, ³Osaka Univ Grad Sch Med). ATF6 is important under both pathological and physiological states in the heart. J Mol Cell Cardiol 2010; 49: 113–20.

Toko H¹, Takahashi H¹, Kayama Y, Oka T^{1,2}, MinaminoT¹, Okada S¹, Morimoto S³, Zhan DY³, Terasaki F⁴, Anderson ME⁵, Inoue M⁶, Yao A⁶, Nagai R⁶, Kitaura Y⁴, Sasaguri T³, Komuro I^{1,2} (¹Chiba Univ Grad Sch Med, ²Osaka Univ Grad Sch Med, ³Kyushu Univ Grad Sch Med, ⁴Osaka Med Coll, ⁵Univ Iowa, ⁶Univ Tokyo Grad Sch Med). Ca2+/calmodulin-dependent kinase II causes heart failure by accumulation of p53 in dilated cardiomyopathy. *Circulation* 2010; **122**: 891–9.

Seki S, Tsutsui K, Fujii T, Yamazaki K, Anzawa R, Yoshimura M. Association of uric acid with risk factors for chronic kidney disease and metabolic syndrome in pataients with essential hypertension. *Clin Exp Hypertens* 2010; **32**: 270-7.

JCS Joint Working Group. Guidelines for diagnosis and treatment of patients with vasospastic angina (coronary spastic angina) (JCS 2008): digest version. *Circ J* 2010; **74:** 1745-62.

Matsuo S, Yamane T, Date T, Hioki M, Ito K, Narui R, Tanigawa S, Nakane T, Hama Y, Tokuda M, Yamashita S, Aramaki Y, Inada K, Shibayama K, Miyanaga S, Yoshida H, Miyazaki H, Abe K, Sugimoto K, Taniguchi I, Yoshimura M. Comparison of the clinical outcome after pulmonary vein isolation based on the appearance of adenosine-induced dormant pulmonary vein conduction. *Am Heart J* 2010; **160**: 337-45.

Harada E¹, Yasue H¹, Mizuno Y¹, Morikawa Y¹, Nakagawa H², Semba F³, Yoshimura M, Saito Y² (¹Kumamoto Aging Res Inst, ²Nara Med Sch, ³Semba Med Clin). Resistant hypertension in an aged woman presenting with clinical features simulating ectopic ACTH syndrome response to spironolactone—. Intern Med 2010; **49:** 2235-9.

Inoue T, Kawai M, Nakane T, Nojiri A, Minai K, Komukai K, Ogawa T, Hongo K, Matsushima M, Yoshimura M. Influence of low-grade inflammation on plasma B-type natriuretic peptide levels. Intern Med 2010; **49**: 2659–68.

Tokuda M, Yamane T, Matsuo S, Ito K, Narui R, Hioki M, Tanigawa SI, Nakane T, Yamashita S, Inada K, Shibayama K, Miyanaga S, Yoshida H, Miyazaki H, Date T, Yokoo T, Yoshimura M. Relationship between renal function and the risk of recurrent atrial fibrillation following catheter ablation. *Heart* 2011; **97**: 137-42.

Ishikawa T¹, Mutoh M¹, Nakano Y¹, Endo A¹, Kubota T, Suzuki T, Nakata K¹, Murakami A, Miyamoto T, Sakamoto H, Okada H, Imai K, Yoshimura M ('Saitama Pref Cardiovasc Resp Ctr). Retrospective comparison of clinical and angiographic outcomes after primary stenting using sirolimus-eluting and bare-metal stents in nonrandomized consecutive 568 patients with first ST-segment elevated myocardial infarctions. J Cardiol 2011: **57**: 44-52.

Matsuo S, Yamane T, Date T, Lellouche N (Henri Mondor Hosp), Tokutake K, Hioki M, Ito K, Narui R, Tanigawa S, Nakane T, Tokuda M, Yamashita S, Aramaki Y, Inada K, Shibayama K, Miyanaga H, Abe K, Sugimoto K, Taniguchi I, Yoshimura M. Dormant pulmonary vein conduction induced by adenosine in patients with atrial fibrillation who underwent catheter ablation. Am Heart J 2011; 161: 188-96.

Aritomi S¹, Wagatsuma H¹, Numata T², Uriu Y², Nogi Y¹, Mitsui A¹, Konda T¹, Mori Y², Yoshimura M ('Ajinomoto Phar, ²Kyoto Univ). Expression of N-type calcium channels in human adrenocortical cells and their contribution to corticosteroid synthesis. *Hypertens Res* 2011; **34**: 193-201.

JCS Joint Working Goup. Guidelines for diagnosis and treatment of myocarditis (JCS 2009): digest version. *Circ J* 2011; **75**: 734-43.

Kubota T^I, Ishikawa T^I, Nakano Y^I, Endoh A^I, Suzuki T^I, Sakamoto H^I, Hasuda T^I, Imai K^I, Yoshimura M, Mutoh M^I (^ISaitama Pref Cardiovasc Resp Ctr). Retrospective comparison of clinical and angiographic outcomes after sirolimus-eluting and bare-metal stents implantation in 312 for consecutive, nonrandomized severely calcified lesions using a rotablator. Int Heart J 2011; 52: 65-71.

Matsuo S, Yamane T, Hioki M, Tanigawa S,

Tokutake K, Ito K, Narui R, Nakane T, Tokuda M, Yamashita S, Inada K, Date T, Sugimoto KI, Yoshimura M. Identification of a conduction gap of the mitral isthmus by using a novel highdensity mapping catheter. *Pacing Clin Electrophysiol* Epub 2010 Oct 28.

Department of Internal Medicine Division of Diabetes, Metabolism and Endocrinology

Kazunori Utsunomiya, Professor Junichi Yokoyama, Professor Katsuyoshi Tojo, Professor Yutaka Mori, Professor Masami Nemoto, Associate Professor Rimei Nishimura, Associate Professor Yoichi Sakamoto, Professor Takashi Sasaki, Professor Kuninobu Yokota, Professor Hideaki Kurata, Associate Professor Tamotsu Yokota, Associate Professor Shuichi Kato, Assistant Professor

General Summary

Physicians should practice patient-oriented medicine based on the concept of evidencebased 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 their reduced regenerative capacity are novel therapeutic targets in the pathophysiology of diabetes are particular our interests in this field. Our study group has succeeded in the direct *in vivo* transfer of genes, including cell-cycle regulators, such as cyclin-dependent kinase 4. We confirmed that regulated proliferation of mature beta cells restores glucose metabolism in diabetic mice.

We have also begun to focus on peri-islet Schwann (PIS) cells to investigate their critical role in the protection of islet endocrine cells and islet development in fetal mice. By using developmental engineering techniques in mice, we found that PIS cells are derived from the neural crest. We also found that PIS cells regulate islet conformation and, probably, alpha/beta cell function via direct contact with alpha cells.

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 compared with a high-carbohydrate enteral formula in patients with type 2 diabetes mellitus and in healthy subjects. By using continuous glucose monitoring, we found that a highly monounsaturated eternal formula significantly suppressed postprandial hyperglycemia and markedly reduced 24-hour glycemic variation in patients with type 2 diabetes receiving tube feeding to a greater extent than did a highcarbohydrate eternal formula, even if carbohydrate nutrients were adjusted to have a low glycemic index.

Diabetic vascular complications

We investigated the role of the Rho/Rho-kinase signaling pathway in the development of diabetic complications. We showed that the Rho/Rho-kinase signaling pathway plays a crucial role in macrophage accumulation in the kidney under diabetic conditions. We also demonstrated that Rho/Rho-kinase mediates thrombin-induced endothelial activation. Intriguingly, we found that the Rho/Rho-kinase signaling pathway is involved in the pathogenesis of diabetic neuropathy. These findings indicate that Rho/Rho-kinase is an important factor that links microvascular complications with macrovascular complications. We are now investigating molecular mechanisms underlying these observations.

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 also examined the differences indicating the stem properties between CD133(+) cells and CD133(-) cells. The effects of Ca^{2+} -channel antagonists on the expression of steroidgenic enzymes were evaluated using human adrenocortical carcinoma cell line NCI-H295R.

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

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

A previous study has shown that ACTH secretion from the hearts of patients with hypertension is increased and suggest that ACTH is involved in the pathophysiology of cardiovascular diseases. Recently, pro-opiomelanocortin messenger RNA has been found to be expressed in the murine heart. Therefore, we designed a study to clarify the pathophysiological role of pro-opiomelanocortin using HL-1 cardiomyocytes.

Publications

Nishimura R, Tsujino D, Taki K, Morimoto A, Tajima N. Continuous glucose monitoring with Humalog Mix 25 versus Humalog Mix 50, twice daily: a comparative pilot study-results from the
Jikei-evaluation of insulin lispro mixture on pharmacodynamics and glycemic Variance (J-EVOLVE) study. *Cardiovasc Diabetol* 2010; **9:** 16.

Ochiai H, Shirasawa T, Nishimura R, Morimoto A, Shimada N, Ohtsu T, Kujirai E, Hoshino H, Tajima N, Kokaze A. Relationship of body mass index to percent body fat and waist circumference among schoolchildren in Japan the influence of gender and obesity: a populationbased cross-sectional study. *BMC Public Health* 2010; **10**: 493.

Shirasawa T, Shimada N, Ochiai H, Ohtsu T, Hoshino H, Nishimura R, Morimoto A, Tajima N, Kokaze A. High blood pressure in obese and nonobese Japanese children: blood pressure measurement is necessary even in nonobese Japanese children. *J Epidemiol* 2010; **20:** 408-12.

Taki K, Nishimura R, Morimoto A, Tsujino D, Miyashita Y, Tajima N. Analysis of 24-hour glycemic excursions in patients with type 1 diabetes by using continuous glucose monitoring. *Diabe*tes Technol Ther 2010; **12**: 523-8.

Matoba K, Kawanami D, Ishizawa S, Kanazawa Y, Yokota T, Utsunomiya K. Rho-kinase mediates TNF-alpha-induced MCP-1 expression via p38 MAPK signaling pathway in mesangial cells. *Biochem Biophys Res Commun* 2010; 402: 725-30.

Department of Internal Medicine Division of Clinical Oncology/Hematology

Keisuke Aiba, Professor Fumi Mizorogi, Professor Daisuke Inoue, Associate Profesor Nobuaki Dobashi, Assistant Professor Kaichi Nishiwaki, Assistant Professor Tadashi Kobayashi, Professor Noriko Usui, Associate Profesor Takaki Shimada, Assistant Professor Hidekazu Masuoka, Assistant Professor Shingo Yano, Assistant Professor

General Summary

The immediate goals of our clinical and basic research are to investigate basic and clinical aspects of malignant diseases and to try to improve outcomes for patients with solid tumors and hematological malignancies, leading to the ultimate goals of improving the natural history of malignant diseases. We have also been performing several clinical trials and basic research studies throughout 2010.

Research Activities

Leukemias

Many patients with previously untreated hematological disorders have been referred to our department. The disorders in 2010 included acute myeloid leukemia (AML), 10 cases; acute lymphoblastic leukemia (ALL), 6 cases, chronic myeloid leukemia (CML), 8 cases; and myelodysplastic syndrome (MDS), 6 cases. We have performed clinical trials as a member of the Japan Adult Leukemia Study Group (JALSG), which is a distinguished leukemia research group established more than 20 years ago in Japan for AML, ALL, and CML. The JALSG protocol studies performed in 2010 were as follows: the AML/MDS-HR CS-7 study for newly diagnosed AML; refractory anemia with excess blasts II, all-case registration cohort study; APL-204 (phase III); CML-207 (phase III); AML-209-GS; and AML209-KIT. We also participated in several cooperative group studies and performed pilot studies: Aged Double-7 (newly diagnosed AML in elderly patients: phase II), VEGA (MDS: phase II), a study of nilotinib (refractory CML: phase I/I), and a study of dasatinib (refractory CML: phase I/I).

Lymphomas

In 2010 we registered 82 patients with newly diagnosed non-Hodgkin's lymphoma and 2 patients with Hodgkin's lymphoma. We have performed clinical trials as a member of the Lymphoma Study Group of the Japan Clinical Oncology Group. Pivotal protocol studies in 2010 were JCOG0406 (newly diagnosed mantle-cell lymphoma: phase II) and JCOG0601 (newly diagnosed low-risk, advanced, diffuse large B-cell lymphoma: phase II/III. A randomized phase II study in patients with high-risk, diffuse, large B-cell lymphoma has also been started (biweekly rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone [R-CHOP] \pm cyclophosphamide, cytarabine, dexamethasone, etoposide, rituximab [CHASER] versus melphalan, cyclophosphamide, etoposide,

and dexamethasone [LEED]; JCOG0908). Other cooperative studies examined biweekly rituximab, etoposide, prednisone, vincristine, hydroxydaunorubicin (R-EPOCH: relapsed and refractory B-cell lymphoma: phase II) and pirarubicin, cyclophosphamide, vincristine, and prednisolone (THP-COP: newly diagnosed T-cell lymphoma: phase II). A study of enzastaurin (non-Hodgkin's lymphoma: phase III double-blind) has been completed. Enzastaurin is a novel drug targeting protein kinase C β which 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 in 2010. A novel agent, the proteasome inhibitor bortezomib, became available in 2007, and we have used it with or without dexamethasone to treat patients who have refractory myeloma. A randomized phase II study was performed to compare the efficacy of bortezomib + dexamethasone with that of thalidomide + dexamethasone in patients with relapsed or refractory chemoresistant multiple myeloma (JCOG0904).

Hematopoietic stem cell transplantation

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

Solid tumors

Many patients with solid cancers have been referred to our department from related divisions or departments from both inside and outside our hospital. Several of our studies seeking improved therapeutic outcomes are in progress throughout our university hospital with related divisions or departments. The combination of fluorouracil, 500 mg/m²; epirubicin, 100 mg/m²; and cyclophosphamide, 500 mg/m² (FEC100) with or without taxotere 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. Doxorubicin and taxotere followed by taxotere and trastuzumab is a first-line chemotherapy for patients with advanced metastatic breast cancer. We have been investigating a combined-modality therapy of radiation and chemotherapy with docetaxel, cisplatin, and 24 hours' continuous infusion of fluorouracil (DCF regimen) for patients with locally advanced esophageal cancer since late The study has been completed, and an improved protocol was launched this 2008. year. A novel drug-development study with an orally decaying formulation of S-1 has been performed for patients with advanced gastric cancer. Our first-line chemotherapies for patients with advanced colorectal cancer are folinic acid, fluorouracil, and oxaliplatin (FOLFOX) and folinic acid, fluorouracil, and irinotecan (FOLFIRI). Because antibodies against vascular endotherial growth factor and epidermal growth factor receptor became available in 2007 and 2008, respectively, combination therapies of these antibodies with

FOLFOX or FOLFILI 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 aims to improve the control of cancer pain. In our new division, we aim to attain individual goals by sharing our thoughts and to contribute to the further growth of palliative care at The Jikei University Hospital.

Basic research

One of our important activities is translational research on solid cancers and hematological malignancies. The structural differences between M protein produced by myeloma cells and that from monoclonal gammmopathy of undetermined significance have been examined, and the function of ATP-binding cassette transporters in cancer chemotherapy has been studied in collaboration with the Keio University Department of Pharmacy. Transfer of the MDR1 gene into hematopoietic stem cells is a potential method of chemoprotection in cancer chemotherapy. Basic research using CD34⁺ cells enables us to try such a strategy. The growth and differentiation of CD34⁺ cells into which the MDR1 gene has been transferred have been investigated *in vitro* in collaboration with the Keio University Department of Pharmacy, and the results have recently been published.

Publications

Nagasaki E, Takahara A, Koido S, Sagawa Y, Aiba K, Tajiri H, Yagita H, Homma S. Combined treatment with dendritic cells and 5-fluorouracil elicits augmented NK cell-mediated antitumor activity through the tumor necrosis factoralpha pathway. J Immunother 2010; **33**: 467-74. Minami J, Dobashi N, Asai O, Yano S, Osawa H, Takei Y, Yahagi Y, Takahara S, Ogasawara Y, Yamaguchi Y, Kobayashi T, Morikawa N, Nikaido T, Aiba K, Usui N. Two cases of mediastinal gray zone lymphoma. J Clin Exp Hematop 2010; **50**: 143-9.

Usui N, Dobashi N, Yano S, Yahagi Y, Takei Y, Otsubo H, Takahara S, Yamaguchi Y, Saito T, Minami J, Kamiyama Y, Morikawa N, Machishima T, Osawa H, Aiba K. High-dose methotrexate followed by whole-brain irradiation for primary central nervous system lymphoma patients – a retrospective study in a single institute(in Japanese). Gan To Kagaku Ryoho 2010; **37:** 1277-82.

Mitsuhashi J, Hosoyama H, Tsukahara S, Katayama K, Noguchi K, Ito Y, Hatake K, Aiba K, Takahashi S, Sugimoto Y. In vivo expansion of MDR1-transduced cells accompanied by a post-transplantation chemotherapy regimen with mitomycin C and methotrexate. *Gene Med* 2010; **12**: 596–603.

Department of Internal Medicine Division of Respiratory Diseases

Kazuyoshi Kuwano, Professor Katutoshi Nakayama, Associate Professor Jun Araya, Assistant Professor Akira Kojima, Associate Professor Masamichi Takagi, Assistant Professor

General Summary

We perform 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 establish novel treatments. We started clinical research concerning COPD in collaboration with the Department of Cardiology and the Department of Diabetes, Metabolism, and Endocrinology. Basic research focused on the molecular mechanisms of lung injury, fibrosis, and COPD. We specifically investigated the roles 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 performed. 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 speculated that early intervention against COPD can prevent various comorbidities. We found that the prevalence of COPD in patients with coronary artery diseases, heart failure, or diabetes mellitus was higher than that in control subjects. Serum levels of tumor necrosis factor and C-reactive protein were decreased in patients treated with statins. Urine levels of hydroxydeoxyguanosine in patients with COPD were higher than in other groups. Clinical research is underway concerning the effects on comorbidities of the treatment of COPD, cardiovascular diseases, or diabetes mellitus.

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. We used human bronchial epithelial primary culture cells and found that insulin was required to protect these cells from apoptosis induced by polyinosinic: polycytidylic acid. Apoptotic signals were dependent on caspase-8 activation. We also found that survival signals were mediated mainly through extracellular signal regulated kinase and Akt activation, although other survival signals may be also associated. We suggest that

insulin administration is a promising strategy 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 have identified senescence as a risk factor for the development of IPF. Among the members of the nicotinamide adenine dinucleotidedependent deacetylase sirtuin (SIRT) family, 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 IPF lungs show enhanced senescence with a concomitant increase in SIRT6 expression in epithelial cells, including aberrantly re-epithelialized bronchial cells. Transforming growth factor (TGF) β induces senescence by increasing p21 expression and also induces SIRT6 expression, and artificial overexpression of SIRT6 efficiently inhibits TGF-β-induced senescence via proteasomal degradation of p21 in human bronchial epithelial cells. Secretion of IL- β 1 by human bronchial epithelial cells with TGF-β-induced senescence is responsible for myofibroblast differentiation in fibroblasts. These findings shed light on the accelerated epithelial senescence in the pathogenesis of IPF and a possible regulatory role for SIRT6.

Autophagy and bronchiolar epithelial cells

To investigate the significance of autophagy in lung diseases, we examined the association between autophagy and the senescence of bronchial epithelial cells. Cigarette smoke extract had numerous effects on bronchial epithelial cells: it induced senescence, transiently upregulated and then downregulated autophagy, and increased missfolded protein and ubiquitinated proteins. However, autophagy digested these unnecessary proteins and protected bronchial epithelial cells from senescence. Our results suggest that autophagy plays important roles in maintaining homeostasis in lung epithelial cells. We are also investigating autophagy in pulmonary fibrosis.

Pulmonary infection

We have started to investigate biomarkers for infectious lung diseases. This study focused on the significance of procalcitonin in the diagnosis and treatment of pulmonary infection.

Lung cancer

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

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. A

manuscript is being prepared for publication.

Publications

Fukumoto J, Harada C, Kawaguchi T, Suetsugu S, Maeyama T, Inoshima I, Hamada N, Kuwano K, Nakanishi Y. Amphiregulin attenuates bleomycin-induced pneumopathy in mice. Am J Physiol Lung Cell Mol Physiol 2010; 298: L131-8.

Minagawa S, Araya J, Numata T, Nojiri S, Hara H, Yumino Y, Kawaishi M, Odaka M, Morikawa T, Nishimura SL, Nakayama K, Kuwano K. Accelerated epithelial cell senescence in IPF and the inhibitory role of SIRT6 in TGF-β-induced senescence of human bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2011; **300:** L391-401.

Harada C¹, Kawaguchi T¹, Ogata-Suetsugu S¹, Yamada M¹, Hamada N¹, Maeyama T¹, Souzaki R¹, Tajiri T¹, Taguchi T¹, Kuwano K, Nakanishi Y¹ (¹Kyushu Univ). EGFR tyrosine kinase inhibition worsens acute lung injury in mice with repairing airway epithelium. Am J Respir Crit Care Med 2011; **183**: 743-51.

Department of Internal Medicine Division of General Medicine

Ken Hokkyo, Professor Norio Tada, Professor Hideaki Suzuki, Associate Professor Hiroshi Yoshida, Associate Professor Jun Hiramoto, Associate Professor Hidekatsu Yanai, Assistant Professor Nobuakira Takeda, Professor Akihiro Nishiyama, Associate Professor Masami Nemoto, Associate Professor Nobuyuki Furutani, Associate Professor Chihiro Shikata, Assistant Professor Takanori Ebisawa, Assistant Professor

Research Activities

Division of General Medicine, The Jikei University Hospital

We are constructing a database of our medical examinations and treatments performed for primary care in outpatients units. The data and information of every outpatient are being gathered from a form of our own design filled out by the physicians. The data and information include the reason for visiting the clinic, physical symptoms or complaints, whether other physicians had been consulted, primary diagnoses, and the contents of examinations and care. The accumulation of our data, especially in the case of initial visits, are expected to be useful for analyzing trends in primary care at major general hospitals.

Division of General Medicine, The Jikei University Aoto Hospital

The mechanisms of the beneficial effects of sarpogrelate, a 5-HT(2A) receptor antagonist, were examined in diabetes mellitus. Sarpogrelate may improve cardiac function in diabetes by promoting the expression of membrane glucose transporters and by releasing insulin from the pancreas.

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 relation of infection with the nutritional state, administered drugs, and biochemical markers. We found that the poor nutritional state and the use of gastric acidsuppressive drugs promote infection in elderly inpatients.

2. Study of fever of unknown origin

We attempted to clarify the causes 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 diagnosing Gramnegative rod sepsis.

Division of General Medicine, The Jikei University Kashiwa Hospital

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

We carried out our tasks of community-based health care according to a regional medical plan in close coordination with the concerned government agencies and with the Kashiwa Medical Society. We developed a new regional nutritional education system for subjects with metabolic syndrome in the Kashiwa area, and this work received a grant from the Chiba Foundation for Health Promotion & Disease Prevention in 2010. We also participated again this year in the development of a local health care system in Kashiwa as members of a local governance committee and recognized the importance of point-of-care testing to detect patients in the early stage of illness. Therefore, we developed a system for measuring lipids in point-of-care testing and reported the results at the 79th annual conference of the European Atherosclerosis Society.

2. Studies of lipid metabolism and atherosclerosis

1) The relationship between the Japanese diet and the incidence of cardiovascular disease was investigated exhaustively through large-scale cohort studies in Japan.

2) Effects of carbohydrate cofeeding with lipids on postprandial hyperlipidemia were investigated with measurement of the serum level apolipoprotein B48.

3) An incubation study with bacteriophages was performed to examine the antiviral effects of plasma fractions, and the antiviral fraction was extracted from human plasma.

4) We developed a method for measuring lipoprotein A with our newly developed highperformance liquid chromatography technique. By measuring very low density lipoprotein cholesterol with this method, we proved the benefit of therapeutic exercise for reducing remnant lipoproteins.

5) Subanalysis by gender of the findings of the Japanese Investigation of Kinetic Evaluation in Hypertensive Event And Remodeling Treatment (JIKEI HEART) study was published.

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

3. Medical Education

1) A new, practical, evidence-based medicine algorithm on clinical practice was developed.

2) As an effective feedback system on undergraduate and postgraduate medical education, a portfolio education system was developed.

Publications

Yoshida H, Shimizu M, Ikewaki K, Taniguchi I, Tada N, Yoshimura M, Rosano G, Dahlöf B, Mochizuki S; Jikei Heart Study group. Sex differences in effects of valsartan administration on cardiovascular outcomes in hypertensive patients: findings from the Jikei Heart Study. J Hypertens 2010; 28: 1150-7.

Das S, Babick AP, Xu YJ, Takeda N, Rodriguez-Levya D, Dhalla NS. TNF-alpha-mediated signal transduction pathway is a major determinant of apoptosis in dilated cardiomyopathy. J Cell Mol Med 2010; 14: 1988-97. Yoshida H, Ishikawa T, Suto M, Kurosawa H, Hirowatari Y, Ito K, Yanai H, Tada N, Suzuki M. Effects of supervised aerobic exercise training on serum adiponectin and parameters of lipid and glucose metabolism in subjects with moderate dyslipidemia. J Atheroscler Thromb 2010; **17**: 1160-6.

Hirowatari Y, Yoshida H, Kurosawa H, Shimura Y, Yanai H, Tada N. Analysis of cholesterol levels in lipoprotein(a) with anion-exchange chromatography. J Lipid Res 2010; **51:** 1237-43. **Tsubota A, Fujise K, Namiki Y, Tada N.** Peginterferon and ribavirin treatment for hepatitis C virus infection. *World J Gastroenterol* 2011; **17:** 419-32.

Wohlschlaeger J, Meier B, Schmitz KJ, Takeda N, Vahlhaus C, Levkau B, Stypmann J, Schmid C, Wermer-Schmid K, Baba HA. Cardiomyocyte survivin protein expression is associated with cell size and DNA content in the failing human heart and is reversibly regulated after ventricular unloading. J Heart Lung Transplant 2010; 29: 1286-92. *Fujise K, Tatsuzawa K, Kono M, Hoshina S, Tsubota A, Niiya M, Namiki Y, Tada N, Tajiri H.* A mutation of the start codon in the X region of hepatitis B virus DNA in a patient with non-B, non-C chronic hepatitis. *World J Hepatol* 2011; **3**: 56-60.

Reviews and Books

Takeda N. Cardiac disturbances in diabetes mellitus. *Pathophysiology* 2010; **17:** 83-8.

Department of Psychiatry

Kazuhiko Nakayama, Professor Kei Nakamura, Professor Hironari Sue, Associate Professor Wataru Yamadera, Assistant Professor Kazuya Ono, Assistant Professor Toshihiko Hashizume, Assistant Professor Rieko Shioji, Assistant Professor Ayumi Tateno, Assistant Professor Hiroshi Itoh, Professor Hisatsugu Miyata, Associate Professor Kazutaka Nukariya, Associate Professor Motohiro Ozone, Assistant Professor Daisuke Ishiguro, Assistant Professor Keita Ohbuchi, Assistant Professor Tadahisa Sannomiya, Assistant Professor

General Summary

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

Research Activities

Psychopathology, psychotherapy, and child study group

Studies examined the care systems for developmental disorders in the psychiatry unit and the difference between "attention deficit" observed in developmental disorders and that observed in other mental disorders. A new counseling method, the Self-Psychological Psychotherapeutic Approach, developed to improve and maintain self-esteem in patients with developmental disorders, was studied, and a clinical trial of it has already been started. A study of basic factors in 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

"Guidelines for Outpatient Morita Therapy" was translated into Chinese. Studies continued this year included those on such topics as the relationship between panic disorder and generalized anxiety disorder, the subtypes of obsessive-compulsive disorder, a "fall-in reaction" occurring in the process of mood or anxiety disorders, and the factors in the recovery of inpatients receiving Morita therapy for depression. A qualitative study of the life histories of female patients with obsessive-compulsive disorder has been performed. In addition, comparative studies between Morita Therapy and third-generation cognitive-behavioral therapies, such as acceptance and commitment therapy, were started.

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 included: 1) research on changes in sleep structures and cognitive function by the menstrual cycle in females using the cyclic alternating pattern method, 2) multi-institutional joint research regarding the development of a novel platform on a website for sleep medicine and research, 3) the development of skills for the diagnosis and treatment of insomnia, 4) empirical research regarding the efficacy of group cognitive behavioral therapy for insomnia on chronic sleep disturbance and depression, and 5) clinical research using the multiple sleep latency for hypersomnias of central origin

Psychogeriatric group

First, a study of the neuropsychological evaluation of neurodegenerative disorders using brain imaging modalities, such as magnetic resonance imaging and single-photon emission computed tomography, suggested that a reduction in hippocampal volume in Alzheimer's disease is related to delayed responses on neuropsychological tasks. Second, an epidemiological survey conducted in Itoigawa City showed no differences in the mortality rates of patients with dementia, although patients with vascular dementia incurred higher costs under the Long-Term Care insurance system than did patients with Alzheimer's disease.

Third, a longitudinal study of the prevalence of psychiatric disorders in patients with breast cancer was performed in collaboration with the Department of Surgery.

General hospital psychiatry

In a study of interventional therapy based on cognitive-behavioral therapy aimed at preventing recurrences of depression, a computer system and sleep evaluation methods were introduced in addition to a previous evaluation system for more effective presentations and for more precise estimation, respectively. Furthermore, an investigation of new indications for this intervention for atypical depression, bipolar depression, and insomnia was performed. Another study investigated the issues associated with mental care services for patients with cancer, 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 several forms of psychosis in patients with epilepsy; and, 2) a prevention study of recurrent depression in epilepsy. Furthermore, we reported on a patient with epilepsy and ring chromosome 20 syndrome and an adult patient with epilepsy and ictal apnea during sleep.

Clinical psychology group

Studies examined: 1) psychotherapeutic processes and the techniques of cognitive behavior therapy, Morita Therapy, relief care, and psycho-oncology and psychiatric care during natural disasters; 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.

Publications

Miyata H, Itasaka M (Senshu Univ), Kimura N, Nakayama K. Decreases in brain reward function reflect nicotine- and methamphetamine-withdrawal aversion in rats. *Curr Neuropharmacol* 2011; **9:** 63-7.

Ito T, Shimizu K, Ichida Y, Ishibashi Y, Akizuki N, Ogawa A, Fujimori M, Kaneko N, Ueda I, Nakayama K, Uchitomi Y. Usefulness of pharmacist-assisted screening and psychiatric referral program for outpatients with cancer undergoing chemotherapy. *Psychooncology* 2011; **20:** 647-54. Epub 2011 Mar 7.

Nakamura K. Formal Discussion: A comment on "Geriatric psychiatric care in Taiwan": Focusing on elder suicide in East Asia. *Int Med J* 2010; **17** *Suppl1*: 37–9.

Reviews and Books

Nakamura K, Hinoguchi J, Tanii K. Psychotherapy for refractory depression: From the standpoint of yojo-ron (taking care of oneself) based on Morita therapy (in Japanese). *Seishinryoho* 2010; **36:** 590-5.

Nakamura K. Recovery process of depression and ant-depressants (in Japanese). *Kokoro no Rinsho a la carte* 2011; **30**: 83-8. Nakamura K. Psychotherapies for social anxiety disorder (in Japanese). Seishinka 2010; **17:** 149-55.

Miyata H. Nicotine: Drug dependence (in Japanese). *Nihon Rinshou* 2010; **68:** 1501-5.

Miyata H. New psychotropic drugs (in Japanese). *Kokoro to Shakai* 2010; **41:** 118-23.

Kubota M, Nakamura K. The initial interview of Morita therapy (in Japanese). *Seishinryoho* 2010; **36:** 478-84.

Nakamura K. Psychotherapy for anxiety (in Japanese). In: Shimoda K, editor. Primary care in psychiatry and brain science 1: Depression and anxiety. Tokyo: Shinajii; 2010. p. 425-34.

Nakamura K. Neurotic disorders and stressrelated disorders (in Japanese). In: Nakayama K, editor. CHART Psychiatry. Revised 4th ed. Tokyo: Igaku Hyoronsha; 2010. p. 222-37.

Nakamura K. Morita therapy for prolonged depression (in Japanese). In: Ohno Y, editor. Handbook for depression treatment. Tokyo: Kongo Shuppan; 2011. p. 277-85.

Nakamura K. Contemporary depression: From a viewpoint of environmental factors (in Japanese). In: The 7th Symposium of Japan Psychiatrists Network on Depression and Anxiety. Tokyo: Arta Shuppan; 2010. p. 37-44.

Department of Pediatrics

Hiroyuki Ida, Professor Yasutaka Hoshi, Professor Takashi Kaneko, Associate Professor Kiyoshi Ogawa, Associate Professor Toshio Katsunuma, Associate Professor Yasuyuki Wada, Associate Professor Kazue Saito, Associate Professor Yoko Kato, Assistant Professor Akinori Syukuya, Assistant Professor Hiroshi Tachimoto, Assistant Professor Yuichi Fuyama, Assistant Professor Takashi Urashima. Assistant Professor Nobuo Usui, Professor Tohya Ohashi, Professor Makiko Okuyama, Associate Professor Shin-ichiro Hamano, Associate Professor Ichiro Miyata, Associate Professor Mitsuyoshi Urashima, Associate Professor Masako Fujiwara, Assistant Professor Yoshihiro Saito, Assistant Professor Masaki Shimizu, Associate Professor Hiroshi Kobayashi, Assistant Professor Yuki Yuza, Assistant Professor Masahisa Kobayashi, Assistant Professor

General Summary

We have 9 subspecialty research groups: (1) the Congenital Metabolic Diseases, Endocrinology, 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 final aim of these subspecialty groups is supplying practical benefits to patients and their families through basic and clinical research. To accomplish this purpose, both cooperation and a high motivation for research are needed.

Research Activities

Congenital Metabolic Diseases, Endocrinology, and Medical Genetics Accomplishments of our group this year are as follows.

1. We clarified the minimum required number of donor cells in bone marrow transplantation for lysosomal diseases.

2. We found that the autophagy build-up in lysosomal disease cells was reduced by a chemical chaperon.

3. We developed a novel gene therapy approach using a lentiviral vector.

4. We found that regulatory T cells are essential in inducing tolerance to enzymes in enzyme replacement therapy for lysosomal diseases.

5. We prepared rats with left ventricular heart failure and analyzed the expression patterns of urocortin 2, urocortin 3, and nesfatin 1 in their brains. Furthermore, we compared messenger RNAs of these genes before and after administration of human atrial natriuretic peptide to these rats.

6. We are developing methods for the molecular diagnosis of genetic diseases by means of competitive genomic hybridization arrays and multiple ligation-dependent probe amplification.

Neurology

1. We investigated sequelae of acute encephalopathy in 103 children. Mental retardation was found in 89.3%, higher cortical dysfunction in 77.7%, epilepsy in 68.9%, and motor disturbance in 27.2%. The severity of disabilities due to sequelae was greatest in acute encephalopathy cased by metabolic disorders. Attention deficit and visuospatial disturbance were the main symptoms of higher cortical dysfunction.

2. We reviewed the medical records of 21 patients with neonatal cerebral infarction to evaluate clinical characteristics. Eighteen patients were born at term. The onset of cerebral infarction was within 2 days in 90% of patients. Seizures were found in 52.4% of patients, and respiratory disorders were found in 33.3%. Infarctions occurred more frequently in the left hemisphere and in the territory of the middle cerebral artery (81.0%). Motor impairments were found as sequelae in 8 patients, mental retardation in 5, and epilepsy in 3.

Hematology and Oncology

We investigated the effects and the molecular mechanisms of histone deacetylase (HDAC) inhibitors valproic acid and depsipeptide on ionizing radiation (IR)-induced apoptosis in Y79 and WERI-Rb1 retinoblastoma cells. We found that valproic acid and depsipeptide synergistically enhanced IR-induced apoptosis, associated with activation of caspase-3 and cleavage of poly (ADP-ribose) polymerase in both cell types. Both valproic acid and depsipeptide enhanced IR-induced phosphorylation of histone H2AX on Ser139 preceding apoptosis. Exposure of cells to IR in the presence of valproic acid or depsipeptide induced the accumulation of p53 acetylated at Lys382 and phosphorylated at Ser46 through the reduction of p53 by HDAC inhibitors is a promising new therapeutic target in refractory retinoblastoma.

Infectious Diseases and Immunologic Disorders

We focus on identification of causative pathogen using polymerase chain reaction (PCR) techniques, genetic diagnosis, treatment of primary immunodeficiency syndrome, and analysis of immune response in pediatric rheumatic diseases.

Our research and development are as follows.

1. Rapid identification of causative pathogen on inflammatory diseases using multiplex PCR

- 2. Quantification of herpesviridae genome using real-time PCR
- 3. Molecular analysis of drug resistance genes of bacteria
- 4. Retrovirus gene therapy for X-linked chronic granulomatous disease
- 5. Disease activities and prognosis of juvenile idiopathic arthritis, systemic lupus erythmatosus, and dermatomyositis
- 6. Efficacy and safety of biologic agents against refractory rheumatic diseases

Nephrology

We retrospectively analyzed the long-term outcomes of 82 children (10 with steroid-resis-

tant nephrotic syndrome [SRNS], 35 with steroid-dependent nephrotic syndrome [SDNS], and 37 with infrequently relapsing nephrotic syndrome [IRNS]) who were initially treated with the International Study of Kidney Disease in Children (ISKDC) regimen at Saitama Children's Medical Center. The ISKDC regimen consists of treatment with prednisolone, 60 mg/m²/day, for 4 weeks, followed by alternate day treatment with 40 mg/m² for another 4 weeks. The aims of our study were to identify factors at onset that could predict the pattern of relapse after the initial treatment with the ISKDC regimen and to assess the prognosis and renal histology after long-term ciclosporin A therapy in 31 children. All 6 asymptomatic children, without edema and identified with proteinuria incidentally detected with a urinary screening program, had an extremely favorable clinical course.

An initial remission time of 9 days or more and the interval from the initial therapy to the first relapse were significant predictors of steroid dependency. These findings had a sensitivity and specificity of 100% and 90%, respectively, a positive predictive value of 95%, and negative predictive value of 100%.

In addition, after the introduction of ciclosporin A therapy, steroid therapy could be stopped in 56% of patients with SRNS and 64% of patients with SDNS. However, after ciclosporin A therapy was tapered or stopped, nephrotic syndrome relapsed in most patients (21 of 20; 95%). Of these patients, 80% (16 of 21) again had SDNS, which was treated with ciclosporin A. Ten of the 22 patients treated with ciclosporin A (mean duration, 31.3 months) had chronic nephrotoxicity.

In conclusion, the initial ISKDC regimen is useful for the early prediction of SDNS. When pediatric nephrologists introduce ciclosporin A therapy in children with SDNS, an alternative strategy should be considered after long-term use of the agent.

Cardiology

The Pediatric Cardiology group is interested in both basic and clinical cardiology research to improve treatment outcomes for children with congenital heart conditions. Our study results were presented at annual meetings of the Japan Pediatric Society and the Japan Pediatric Cardiology Society. Grants from 2 national foundations were distributed to our group to study right ventricle heart failure and copy number variant in congenital heart disease. Specific projects under way in our group include the following.

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

We also are interested in specific clinical research projects, as follows.

- 1. Making appropriate management plans based on fetal diagnosis.
- 2. Long-term outcomes in patients with total cavopulmonary connection circulation.
- 3. Interventional catheterization (balloon angioplasty and valvuloplasty, coil emboliza-

tion, transcatheter stenting, and catheter closure of congenital heart defects).

Publications

Kobayashi H, Shimada Y, Ikegami M, Kawai T, Sakurai K, Urashima T, Ijima M, Fujiwara M, Kaneshiro E, Ohashi T, Etoh Y, Ishigaki K, Osawa M, Kyosen SO, Ida H. Prognostic factors for the late onset Pompe disease with enzyme replacement therapy: from our experience of 4 cases including an autopsy case. *Mol Genet Metab* 2010; **100**: 14–9.

Kyosen SO, lizuka S, Kobayashi H, Kimura T, Fukuda T, Shen JS, Shimada Y, Ida H, Eto Y, Ohashi T. Neonatal gene transfer using lentiviral vector for murine Pompe disease: long term expression and glycogen reduction. *Gene Ther* 2010; **17:** 521-30.

Okuyama T, Tanaka A, Suzuki Y, Ida H, Tanaka T, Cox GF, Eto Y, Orii T. Japan Elaprase Treatment (JET) Study. Idursulfase enzyme replacement therapy in adult patients with attenuated Hunter syndrome (Mucopolysaccharidosis) II, MPS II). *Mol Genet Metab* 2010; **99:** 18-25.

Hamano S, Higurashi N, Koichihara R, Oritsu T, Kikuchi K, Yoshinari S, Tanaka M, Minamitani M. Interictal cerebral blood flow abnormality in cryptogenic West syndrome. *Epilepsia* 2010; 51: 1259-65.

Kikuchi K, Hamano S, Goto F, Takahashi A, Ida H. Epileptic focus in a case of subcortical band heterotopia: SISCOM and ictal EEG findings. *Epilepsy & Seizure* 2010; **3:** 192-8.

Higurashi N, Hamano S, Yoshinari S, Tanaka M, Ida H. Nonthalamic generalization of Ictal spikes in atypical absence seizures. *Pediatr Neurol* 2010; **43:** 131-4.

Yamaoka M, Akiyama M, Yuza Y, Yokoi K, Yokokawa Y, Matsushima S, Fujigasaki J, Chiba S, Kohno M, Ida H. Disseminated Absidia corymbifera infection in a 14-year-old girl with Burkitt lymphoma (stage IV). Nippon Shoni Ketsueki Gakkai Zasshi 2010; **24:** 43-6.

Kawano T, Akiyama M, Agawa-Ohta M, Mikami-Terao Y, Iwase S, Yanagisawa T, Ida H, Agata N, Yamada H. Histone deacetylase inhibitors valproic acid and depsipeptide sensitize retinoblastoma cells to radiotherapy by increasing H2AX phosphorylation and p53 acetylation-phosphorylation. Int J Oncol 2010; **37**: 787-95.

Goto-Sugai K, Tsukagoshi H, Mizuta K, Matsuda S, Noda M, Sugai T, Saito Y, Okabe N, Tashiro M, Kozawa K, Tanaka R, Morita Y, Nishina A, Kimura H. Genotyping and phylogenetic analysis of the major genes in respiratory syncytial virus isolated from infants with bronchiolitis. Jpn J Infect Dis 2010; 63: 393-400.

Kawai T, Kusakabe H, Seki A, Kobayashi S, Onodera M. Osteomyelitis due to trimethoprim/ sulfamethoxazole-resistant Edwardsiella tarda infection in s patient with X-linked chronic granulomatous disease. Infection 2011; **39:** 171-3. Epub 2011 Jan 19.

Ito Y, Adachi Y, Itazawa T, Okabe Y, Adachi YS, Katsunuma T, Miyawaki T. Comparison of exhalation time methods (6 sec vs. 10 sec) of a hand-held exhaled nitric oxide analyzer. *Pediatr Pulmonol* 2010; **45:** 1005-8.

Sato S, Tachimoto H, Shukuya A, Kurosaka N, Yanagida N, Utsunomiya T, Iguchi M, Komata T, Imai T, Tomikawa M, Ebisawa M. Basophil activation marker CD203c is useful in the diagnosis of hen's egg and cow's milk allergies in children. Int Arch Allergy Immunol 2010; **152 Suppl 1:** 54–61.

Ebisawa M, Sugizaki C. Establishment of food provocation network in Japan. Environmental and genetic factors in allergy and clinical immunology proceedings of the 27th symposium of the collegium internationale allergologicum. *PACINI EDITORE MEDICINA* 2010: 199–201.

Matsumoto K, Terakawa M, Fukuda S, Saito H. Analysis of signal transduction pathways involved in anti-CD30 mAb-induced human eosin-ophil apoptosis. *Int Arch Allergy Immunol* 2010; **152 Suppl 1:** 2-8.

Teramoto S, Tanabe Y, Okano E, Nagashima T, Kobayashi M, Eto Y. A first fatal neonatal case of Enterobacter sakazakii infection in Japan. *Pediatr Int* 2010; **52:** 312-3.

Fujiwara T, Okuyama M, Takahashi K. Paternal involvement in childcare and unintentional injury of young children: a population-based cohort study in Japan. *Int J Epidemiol* 2010; **39:** 588-97.

Reviews and Books

Saito H. Mast cell-specific genes as new drug targets. In: Pawnkar R, Holgate ST, Rosenwasser LJ, editors. Allergy frontiers: Future perspectives. NY: Springer; 2010. p. 179-90.

Suzukawa M, Yamaguchi M, likura M, Koketsu R, Komiya A, Nagase H, Nakae S, Matsumoto K, Saito H, Matsushima K, Yamamoto K, Ohta K. IL-33-induced activation of human basophils and eosinophils. Inflammation Regenerat 2010; 30: 181-5.

Department of Dermatology

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

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 provide a significant contribution to excellent clinical practice.

Research Activities

Psoriasis

Various systemic therapies, including oral cyclosporin microemulsion preconcentrate, methotrexate, etretinate, topical vitamin D3, and corticosteroids, have been used depending on disease severity and the degree to which quality of life (QOL) is impaired in individual patients. Also phototherapy, including psoralen ultraviolet A, narrow-band ultraviolet B (UVB), and 308-nm exicimer lamp, are effective and have been administered in a newly organized skin-care clinic. We have evaluated patients' QOL reflecting social background and developed a Japanese version of the Psoriasis Disability Index. We also examined the incidence of metabolic syndromes as a comorbidity of psoriasis. In a special psoriasis clinic, we select patient-based treatments to satisfy patients' demands. New biologic agents, including infliximab and adalimumab, are available and have been used to treat intractable psoriasis. Clinical trials have been performed with new biologic agents, including antibodies against interleukin (IL) 17A and IL-23p19. We have organized meetings twice a year with a society of patients with psoriasis in the Tokyo area 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 and host susceptibility genes, altered skin barrier function, and the immune system. Recently, psychosocial factors have been suggested to influence the exacerbation of atopic dermatitis. Therefore, we are treating patients on the basis of not only evidence-based medicine but also on QOL issues. We try to obtain a precise medical history from each patient and to learn how QOL is impaired. To support this type of approach, we have organized skin-care lessons in the Skin-Care Clinic twice a week and have hosted an atopic dermatitis forum, which includes monthly lectures and group meetings. For basic clinical research, the levels of substance P, thymus and activation-regulated cytokine, and IL-31 related to pruritus in atopic dermatitis are being evaluated according to disease severity. Clinical trials of opioid-k agonist have been performed.

Malignant skin tumors

We have been studying clinical courses and postoperative outcomes of patients with malignant melanoma, extramammary Paget's disease, squamous cell carcinoma, basal cell carcinoma, malignant peripheral nerve sheath tumor, malignant fibrous tumors, or 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 because many patients with letters of introduction visit from all over Japan, we concentrate on long-term follow-up and improvement of impaired QOL by means of accurate diagnosis and the resection of neurofibromas. The estimated lifetime risk of malignant peripheral nerve sheath tumor (MPNST) in patients with neurofibromatosis 1 is 10%, although information concerning the epigenetic abnormality is limited. We have used the methylation-specific polymerase chain reaction (PCR) and real-time reverse transcriptase (RT)-PCR to analyze the methylation status of tumor suppressor genes and cancer-testis genes in established MPNST cell lines. The findings of abnormal expression of several cancer-testis genes and the inactivation of tumor suppressor genes indicate that disarranged methylation and 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 varaciclovir 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. Neurological complications are commonly associated with herpes zoster. PHN, defined as pain present for 90 days after the onset of rash, is a major sequela of VZV infection and impairs QOL. To prevent PHN, we proactively use tricyclic antidepressants. Post-hoc analyses of a subgroup of patients showed that amitriptyline in combi-

nation with acyclovir reduced PHN in its incidence. PHN is characterized by various types of pain and sensory symptoms, including ongoing pain, allodynia, and evoked or spontaneous intermittent lancinating pains. We are actively prescribing pregabalin, tricyclic antidepressants, selective serotonin reuptake inhibitors, opioid analgesics, and topical analgesics and are using visual analogue scales and an objective measuring device (Pain Vision PS-2100, Nipro Co., Osaka) to evaluate pain.

Human papillomavirus 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 CO_2 and pulsed dye laser evaporation has also been used to treat severe, intractable viral warts. Human papillomavirus infection 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 accuminatum.

Contact dermatitis/drug eruption

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

Laser

The Q-switched ruby laser is useful for treating nevus Ota 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 CO_2 laser has higher energy and a shorter pulse width, it can vaporize at a fixed depth and can be used to quickly remove actinic keratosis, seborrheic keratosis, syringoma, and epidermal nevus.

Skin Care Clinic

Narrow-band UVB irradiation is performed for patients with psoriasis, atopic dermatitis, prurigo nodularis, vitiligo, or cutaneous T-cell lymphomas. Targeted phototherapy equipment, such as the 308-nm exicimer 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 exicimer 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 inpatients and outpatients to enhance QOL. Genetic analysis was performed for MPNST.

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

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

Contact dermatitis: For causal chemicals, environmental allergens, drugs, and foods in patients with contact dermatitis, drug eruption, patch testing is are regularly performed.

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

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

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.

Publications

Shibata S, Tada Y, Kanda N, Nashiro K, Kamata M, Karakawa M, Miyagaki T, Kai H, Saeki H, Shirakata Y, Watanabe S, Tamaki K, Sato S. Possible roles of IL-27 in the pathogenesis of psoriasis. J Invest Dermatol 2010; **130**: 1034-9.

Nakamura T, Sato Y, Watanabe D, Ito H, Shimonohara N, Tsuji T, Nakajima N, Suzuki Y, Matsuo K, Nakagawa H, Sata T, Katano H. Nuclear localization of Merkel cell polyomavirus large T antigen in Merkel cell carcinoma. *Virology* 2010; **398**: 273–9. Ohmatsu H, Kadono T, Sugaya M, Tomita M, Kai H, Miyagaki T, Saeki H, Tamaki K, Steeber DA, Tedder TF, Sato S. α4β7 Integrin is essential for contact hypersensitivity by regulating migration of T cells to skin. J Allergy Clin Immunol 2010; **126**: 1267-76.

Miyagaki T, Sugaya M, Yokobayashi H, Kato T, Ohmatsu H, Fujita H, Saeki H, Kikuchi Y, Tamaki T, Sato S. High levels of soluble ST2 and low levels of IL-33 in sera of patients with HIV infection. J Invest Dermatol 2011; 131: 794-6. Shibata S, Tada Y, Hau C, Tatsuta A, Yamamoto M, Kamata M, Karakawa M, Asano Y, Mitsui H, Sugaya M, Kadono T, Saeki H, Kanda N, Sato S. Adiponectin as an antiinflammatory factor in the pathogenesis of psoriasis: induction of elevated serum adiponectin levels following therapy. Br J Dermatol 2011; 164: 66770.

Yano C, Nakagawa H, Kono M, Mochizuki T. A pediatric case of kerion celsi caused by Trichophyton tonsurans (NTS type 3) (in Japanese). Nihon Shoni Hifuka Gakkai Zasshi 2010; 29: 131-4.

Department of Radiology

Kunihiko Fukuda, Professor Junta Harada, Professor Yukio Miyamoto, Professor Mayuki Uchiyama, Associate Professor Manabu Aoki, Associate Professor Yoshimitsu Sunagawa, Assistant Professor Takuji Mogami, Assistant Professor Chihiro Kanehira, Professor Toru Sekiya, Professor Shunichi Sadaoka, Associate Professor Hiroya Ojiri, Associate Professor Norio Nakata, Assistant Professor Masao Kobayashi, Assistant Professor

Research Activities

Division of diagnostic imaging

1. Clinical usefulness of diffusion-weighted imaging in patients with rheumatoid arthritis

The clinical usefulness of diffusion-weighted imaging (DWI) in the evaluation of the activity of inflammatory synovitis in patients with rheumatoid arthritis (RA) was assessed in comparison with fat-suppressed contrast-enhanced magnetic resonance imaging (MRI), which was defined as the gold standard. Correlation of 100% was obtained in all 60 regions of interest in 4 patients. Furthermore, DWI can be substituted for fat-suppressed contrast-enhanced MRI in patients in whom administration of contrast media is contraindicated.

2. Evaluation of the ankle joint with MRI and dual-source computed tomography

The combination of MRI and 3-dimensional display using dual-source (DS) computed tomography (CT), is superior to other modalities for evaluating the anatomical and pathological relationships between the tendons and osseous components of the ankle.

3. Coronary CT angiography using DS-CT: Comparison with fractional flow reserve (FFR) using flow wire-coronary CT angiography (CTA) using DS-CT shows high diagnostic accuracy for anatomical stenosis diagnosed with coronary angiography, even for patients with a high heart rate not being treated with a β -blocker, which increases the incidence of side effects of contrast media. For the functional evaluation of coronary artery stenosis, the FFR is measured with 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 prognostic factor in patients with head and neck cancers. We established a CT scoring system that uses the criteria of size, shape, extracapsular spread, and focal defects and assessed its applicability in comparison with surgical specimens of neck dissection.

Division of Nuclear Medicine

1. Multicenter trial confirmed the effectiveness of strontium-89 for palliative pain relief in cases of multiple bone metastases

The bone-seeking radiopharmaceutical Sr-89 has been used as a palliative treatment for

patients with pain caused by metastases to bone. 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. Images of Sr-89 had not been published before our report.

2. Assessment of combination therapy with strontium-89 and iodine-131 for thyroid cancer metastatic to bone

Metastasis to bone in patients with thyroid cancer is an intractable condition. We have used I-131 therapy for thyroid cancer. A suitable protocol should be assessed for combination therapy with Sr-89 and I-131 targeting bone metastases.

Division 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 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.

Division of Radiation Therapy

1. Evaluation of relationship between bronchiolitis obliterans organizing pneumonia and adjuvant therapy (hormonal therapy/chemotherapy) for early breast cancer treatment We have encountered several cases of radiation-induced bronchiolitis obliterans organizing pneumonia syndrome in patients treated for early breast cancer and analyzed the relationship between the development of this syndrome and adjuvant therapy (hormonal therapy/chemotherapy).

2. Evaluation of the efficacy of chemoradiotherapy for local control in patients with local advanced esophageal cancer refractory to docetaxel, cisplatin, and 5-fluorouracil

The triple-drug combination chemotherapy of docetaxel, cisplatin, and 5-fluorouracil (DCF) before surgery is effective for advanced esophageal cancer. However, cases refractory to DCF likely have a poor prognosis. We evaluated the efficacy of chemoradiotherapy DCF for local control in patients with DCF-refractory local, advanced esophageal cancer.

3. Application of intensity-modulated radiotherapy for adjuvant and salvage radiotherapy after radical prostatectomy

Conventional radiotherapy has long been used as an adjuvant and salvage therapy after radical prostatectomy. However, these treatments are not effective enough to cure residual prostate cancer. We evaluate the effectiveness and adverse events of intensity-modulated radiotherapy in which the prostate bed and the pelvic lymph nodes have been irradiated.

Publications

Kurosaka D, Hirai K, Nishioka M, Miyamoto Y, Yoshida K, Noda K, Ukichi T, Yanagimachi M, Furuya K, Takahashi E, Kingetsu I, Fukuda K, Yamada A. Clinical significance of serum levels of vascular endothelial growth factor, angiopoietin-1, and angiopoietin-2 in patients with rheumatoid arthritis. *J Rheumatol* 2010; **37**: 1121-8.

Miyamoto Y, Onoue K, Nishioka M, Nakata N, Matsuura T, Asakura T, Ohkawa K, Tsuchiya K, Itani K, Konno T, Sasaki H, Abe M. Experimental study of the stability of sonazoid. Jikeikai Med J 2010; 57: 55-60.

Zeniya M, Yokoyama K, Imamura N, Murai S, Ishikawa T, Hokari A, Koike K, Takahashi H, Sadaoka S. Significance of interferon-beta for the treatment of hepatitis C virus infection in hemodialyzed patients. *Hepatol Res* 2010; **40**: 862-9.

Kobayashi M, Takagi S, Shirahama J, Aoki M, Sunakawa Y, Honda C, Oowaki K, Nakagawa M, Kanehira C. Clinical evaluation of 5 cases with simultaneous triple cancer (oropharynx, hypopharynx, esophagus) (in Japanese). *Rinsho Hoshasen* 2010; **55:** 1639-45.

Kitaoka M, Miyamoto Y, Fukunari N, Omoto K, Kameyama K, Shimura H, Suzuki S, Miyagawa M, Miyabe R, Murakami T. Draft of ultrasound diagnostic criteria for thyroid nodule (Mass) (in Japanese). Choonpa Igaku 2011; **38**: 27-30.

Yoshida M, Irie T, Inagawa T, Yamashita Y, Takamura K, Yamakawa H, Sadaoka A, Naruo K, Saigusa H, Miyamoto Y. Treatment of iatrogenic femoral artery pseudoaneurysms with USguided low-dose thrombin injection (in Japanese). *Choonpa Igaku* 2011; **38:** 13-7.

Hirai K, Kurosaka D, Nishioka M, Miyamoto Y, Fukuda K, Yamada A. Clinical significance of Wrist joint synovial blood-flow signals on Doppler ultrasonography in patients with rheumatoid arthritis. Jikeikai Med J 2010; **57:** 21–6.

Reviews and Books

Kanehira C. Radiotherapy of bone and soft tissue tumors (in Japanese). *Gan Chiryou Lecture* 2011; **2**: 169–74.

Munakata K, Miyamoto M, Nishioka M, Onoue K, Nakata N. The latest topic of the contrast ultrasonography (in Japanese). *Shin Iryou* 2010; **37:** 156-60.

Aoki M. The definition of Biochemical failure in prostate cancer (in Japanese). In: Kakei Y, editor. Zenritusengan shinryo. Tokyo: Chugai Igakusha; 2010. p. 151-3.

Kanehira C. Bone and Soft Tissue Sarcomas (in Japanese). In: Onishi H, Karasawa K, Karasawa K, editors. Gan houshasenryouhou 2010. Tokyo: Shinohara Shuppann Shinsha; 2010. p. 1035-9.

Department of Surgery Division of Digestive Surgery

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General Summary

The quality of surgical care is defined as the degree to which surgical practice for individuals and populations increases the likelihood of desired health outcomes and are consistent with current professional knowledge. Yet, for much of the 20th century, the knowledge base for surgical practice was not influenced by systematic evidence, but rather by expert opinion and collective experience. Now, evidence-based medicine has been widely accepted in surgery. Randomized clinical trials are not valid sources of evidence to answer a large number of important clinical questions. Well-designed observational research can also address relevant clinical questions that cannot be answered by randomized clinical trials. Some of our studies listed below would help both surgeons and patients to make decisions and would improve the quality of health care in the surgical field.

Research Activities

Upper gastrointestinal surgery

We studied the pathophysiology of esophageal functional disorders, such as esophageal achalasia and gastroesophageal reflux disease, using manometry and multichannel intraluminal impedance pHmetry. We proposed surgical indications for these esophageal functional diseases and performed surgical treatment with laparoscopic surgery. We continue to assess the viability of the gastric tube using intraoperative thermal imaging system during esophagectomy. The correlation between suitable graft construction and postoperative complications of a graft was then investigated. We started intraoperative monitoring of bilateral laryngeal recurrent nerves to prevent postoperative laryngeal research in esophageal cancer has led us to find molecular markers indicating prognosis. We aimed to investigate the significance of the expression of small ubiquitin-like modifier 1 in esophageal cancer as such a prognostic factor. Early gastric cancer invading the submucosa is associated with an approximately 20% risk of lymph node metastasis, for which gastrectomy with lymph dissection is indicated. However, gastric preservation is possible by searching for sentinel lymph nodes, which are assumed to be the lymph nodes to which cancer first metastasizes. We examined the usefulness of sentinel lymph node navigation surgery with the infrared ray endoscope to develop laparoscopic operations that allow submucosal cancers of the stomach to be safely resected.

We also investigated the characteristics, such as malignant potential in the progress of gastric cancer, in relation to the sensitivity to anticancer agents.

Postgastrectomy syndrome occurs after gastrectomy performed to treat gastric diseases, most often gastric cancer. The severity of postgastrectomy syndrome is related mainly to the extent of gastric resection and the reconstruction procedures. Postgastrectomy syndrome is a clinically important problem, because it impairs quality of life. To minimize postgastrectomy syndrome our department has actively pursued function-preserving gastrectomy, reconstruction with a substitute stomach, and limited gastric resection. We have also performed postoperative gastrointestinal function tests to evaluate various gastrectomy procedures and to obtain useful information for the diagnosis or treatment of postgastrectomy syndrome.

Colorectal surgery

To improve the quality of laparoscopic operations, we are evaluating the usefulness and reliability of the Virtual Reality Surgical Simulator for laparoscopic colectomy. A comparative study of stress between open and laparoscopic surgery is under way.

In chemotherapy, we are participating in national multicenter trials to obtain new evidence. Moreover, we are developing a new regimen (S-1, oxaliplatin plus cetuximab, SOX+C) in collaboration with the Division of Oncology/Hematology, Department of Internal Medicine.

There have been no breakthroughs in basic research on various antibodies in relation to cancer. However, the efficacy of indoleamine 2,3-dioxygenase (the enzyme that causes cancer immunotolerance) as a factor predicting the recurrence of early colorectal cancer has been reported. Furthermore, in collaboration with the Department of Urology, we are developing methods to identify cancer-associated proteins (colorectal, esophageal, gastric, pancreatic, and liver cancers) by means of proteomics.

Sclerotherapy with aluminum potassium sulfate and tannic acid has been used to augment the treatment of anorectal diseases. Functional analysis of anorectal function using stationary 3-dimensional manometry has been introduced in Japan, and a systemic treatment strategy for anorectal diseases is expected to be developed.

Hepatobiliary and pancreatic surgery

Our main research activities are as follows.

- 1. Living donor liver transplantation (LDLT) and regenerative medicine
- 2. Treatment for hepatocellular carcinoma and control of recurrence
- 3. Chemotherapy for pancreatic and biliary cancers
- 4. Expansion of surgical indications for multiple hepatic tumors

- 5. Laparoscopic surgery for the liver, biliary tree, pancreas, and spleen
- 6. Navigation surgery for hepatobiliary and pancreatic surgery
- 7. Nutritional therapy for cancer patients
- 8. Surgical site infection control in surgical patients

9. Effect of preoperative treatment with eltrombopag and splenectomy for idiopathic thrombocytopenic purpura

The first LDLT was successfully performed for a patient with postnecrotic cirrhosis and hepatocellular carcinoma on February 9, 2007. Our ninth LDLT was performed for a patient with primary biliary cirrhosis on September 17, 2010. All 9 recipients were discharged on postoperative days 15 to 46 in good condition, and all donors were discharged on postoperative days 9 to 13 and returned to their preoperative status. We are also planning to extend the indications of LDLT to acute hepatic failure and ABO-incompatible cases. We have performed translational research on combination chemotherapy with gemcitabine and a protease inhibitor, FUT-175, which is associated with both nuclear factor κ -B inhibition and apoptosis induction in pancreatic cancer cell lines. Navigation surgery for liver resection is ongoing as an Advanced Medical Treatment from February 1, 2011, and biliary and pancreatic navigation surgery is performed with the Institute for High Dimensional Medical Imaging Research Center. Other continuing clinical and experimental trials involve treatment of hepatic tumor, laparoscopic surgery, nutritional therapy, resection site infection, and eltrombopag as a pretreatment for laparoscopic splenectomy for idiopathic thrombocytopenic purpura.

Publications

Tsuboi K, GazalloJ, Yano F, Filipi CJ, Mittal SK. Good training allows excellent results for laparoscopic Nissen fundoplication even early in the surgeon's experience. *Surg Endosc* 2010; **24:** 2723-9.

Fujiwara Y, Shiba H, Furukawa K, Iida T, Haruki K, Gocho T, Wakiyama S, Hirohara S, Ishida Y, Misawa T, Ohashi T, Yanaga K. Glasgow prognostic score is related to blood transfusion requirements and post-operative complications in hepatic resection for hepatocellula carcinoma. Anticancer Res 2010; **30**: 5129–36.

Ishii Y, Sakamoto T, Ito R, Yanaga K. F2-isoprostanes and 2-arachidonylglycerol as biomarkers of lipid peroxidation in pigs with hepatic ischemia/reperfusion injury. *J Surg Res* 2010; **161**: 139-45.

Kawahara H, Watanabe K, Ushigome T, Noaki R, Kobayashi S, Yanaga K. Umbilical incision laparoscopic surgery with one assist port for anterior resection. *Dig Surg* 2010; **27:** 364-6.

Kawahara H, Watanabe K, Ushigome T, Noaki R, Kobayashi S, Yanaga K. Where is the best skin incision for partial resection of the small intestine? Hepatogastroenterology 2010; 57: 794-6. Furukawa K, Iida T, Shiba H, Fujiwara Y, Uwagawa T, Shimada Y, Misawa T, Ohashi T, Yanaga K. Anti-tumor effect by inhibition of NF- kappaB activation using nafamostat mesilate for pancreatic cancer in a mouse model. *Oncol Rep* 2010; **24:** 843-50.

Kawahara H, Watanabe K, Ushigome T, Naoki R, Kobayashi S, Yanaga K. Retrograde single stapling technique for laparoscopic ultralow anterior resection. *Dig Surg* 2010; **27:** 261-4.

Kelder W, Nimura H, Takahashi N, Mitsumori N, van Dam GM, Yanaga K. Sentinel node mapping with indocyanine green (ICG) and infrared ray detection in early gastric cancer: an accurate method that enables a limited lymphadenectomy. *Eur J Surg Oncol* 2010; **36**: 552-8.

Noaki R, Kawahara H, Watanabe K, Ushigome T, Kobayashi S, Yanaga K. Serum p53 antibody is a useful tumor marker of early colorectal cancer. Int Surg 2010; **95:** 287-92.

Kawahara H, Watanabe K, Ushigome T, Noaki R, Kobayashi S, Yanaga K. Single-incision laparoscopic right colrectomy for recurrent Crohn's disease. *Hepatogastroenterology* 2010; **57**: 1170-2.

Kawahara H, Watanabe K, Ushigome T, Noaki R, Kobayashi S, Yanaga K. Laparoscopyassisted lateral pelvic lymph node dissection for advanced rectal cancer. *Hepatogastroenterology* 2010; **57:** 1136-8.

Ushigome T, Kawahara H, Kobayashi T,

Kobayashi S, Kashiwagi H, Yanaga K. Where should the specimen be excised for the chemosensitivity of colorectal cancer to 5-fluorouracil? *Hepatogastroenterology* 2010; **57:** 1090-4.

Saito R, Ishii Y, Ito R, Nagatsuma K, Tanaka K, Saito M, Maehashi H, Nomoto H, Ohkawa K, Mano H, Aizawa M, Hano H, Yanaga K, Matsuura T. Transplantation of liver organoids in the omentum and kidney. Artif Organs 2010; **35**: 80-3.

Fujiwara Y, Shiba H, Furukawa K, lida T, Sakamoto T, Gocho T, Wakiyama S, Hirohara S, Ishida Y, Misawa T, Ohashi T, Yanaga K. Perioperative change in white blood cell count predicts outcome of hepatic resection for hepatocellular carcinoma. J Hepatobiliary Pancreat Sci 2010; 17: 892-7.

lida T, Shiba H, Misawa T, Ohashi T, Eto Y, Yanaga K. Immunogene therapy against colon cancer metastasis using an adenovirus vector expressing CD 40 ligand. *Surgery* 2010; **148**: 925-35.

Nishikawa K, Yanaga K, Kashiwagi H, Hanyuu N, Iwabuchi S. Significance of intraoperative endoscopy in total gastrectomy for gastric cancer. Surg Endosc 2010; 24: 2633-6.

Hoya Y, Okamoto T, Yanaga K. Evaluation of analgesic effect and safety of fentanyl transdermal patch for cancer pain as the first line. *Support Care Cancer* 2010; **18**: 761-4.

Omura N, Kashiwagi H, Yano F, Tsuboi K, Yanaga K. Postoperative recurrence factors of GERD in the elderly after laparoscopic fundoplication. *Esophagus* 2010; **7**: 31-5.

Hoya Y, Okamoto T, Fujita T, Kashiwagi H, Yanaga K. Reduction of peritoneal drain-related complications after guidelines for drain placement and a novel drain fixation device. *Jikeikai Med J* 2010; **57:** 61–5.

Aoki H, Kushimoto S, Koike K, Shibata Y, Yamamoto Y, Yanaga K. Significance of serum procalcitonin measurement in sepsis due to peritonitis. Jikeikai Med J 2010; 57: 5-10.

Usuba T, Misawa T, Toyama Y, Ishida Y, Ishii Y, Yanagisawa S, Kobayashi S, Yanaga K. Is modified devine exclusion necessary for gastrojejunostomy in patients with unresectable pancreatobiliary cancer? Surg Today 2011; 41: 97-100.

Okamoto T, Shida A, Fujioka S, Hoya Y, Yanaga K. Usefulness of modified devine gastrojejunostomy as a palliative surgery. *Jikeikai Med J* 2010; **57:** 121-6.

Tsuboi K, Mittal SK, Legner A, Yano F, Filipi CJ. Relationship between manometric findings and reported symptoms in nutcracker esophagus: insights gained from a review of 313 patients. *J Gastroenterol* 2010; **45**: 1033-8.

Reviews and Books

Fujita T. Role of mechanical bowel preparation and anastomotic technique in low anterior resection. *Ann Surg* 2011; **253:** 629.

Hoya Y, Taki T, Tanaka Y, Yano F, Hirabayashi T, Okamoto T, Kashiwagi H, Yanaga K. Disadvantage of operation cost in laparoscopyassisted distal gastrectomy under the national health insurance system in Japan. *Dig Surg* 2010; **27:** 343-6.

Fujita T. Risk assessment for recurrent venous thrombosis. *Lancet* 2011; **377:** 1072-3.

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

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General Summary

The Divisions of Chest Surgery, and of Breast and Endocrinology Surgery were established in June 2005. Since then, all staff members have been active in surgical practice, research, and education. Many studies are 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 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 thoracoscopic diagnosis of benign and malignant lung diseases, and LaparoSonic coagulating shears (Ethicon Endo-Surgery, Inc., Cincinnati, OH, USA) for small thoracotomy. Three-dimensional diagnosis with computed tomography is used to make thoracoscopic surgery safer. The diagnosis and treatment of ground glass opacity of the lung, which is considered to indicate early adenocarcinoma, are being evaluated.

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

DCIS has increased to account for 20% of breast cancers with the spread of screening mammography in Japan. We have studied biological factors involved in the progression of DCIS to invasive breast cancer by immune-staining procedures.

We have performed phase II/III studies of contrast-enhanced ultrasonography of the breast with the microbubble contrast medium Sonazoid (Daiichi Sankyo Co. Ltd., Tokyo), in cooperation with the Department of Radiology. With Sonazoid the sensitivity of ultrasonography for the detection of small breast cancers equals that of magnetic resonance imaging.

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

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

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

Publications

Akiba T, Marushima H, Kawahara H, Takagi M, Hirano J, Odaka M, Nakanishi K, Takeyama H, Kobayashi S, Morikawa T. Video-assisted thoracic surgery for patients with lung cancer and interstitial pneumonia. *Ann Thorac Cardiovasc Surg* 2010; **16:** 236-41.

AkibaT, MarushimaH, MorikawaT. Confirmation of a variant lingular vein anatomy during thoracoscopic surgery. Ann Thorac Cardiovasc Surg 2010; **16:** 351-3.

Akiba T, Marushima H, Odaka M, Harada J, Kobayashi S, Morikawa T. Pulmonary vein analysis using three-dimensional computed tomography angiography for thoracic surgery. Gen Thorac Cardiovasc Surg 2010; 58: 331-5.

Odaka M, Akiba T, Nakada T, Asano H, Yabe M, Kamiya N, Hirano J, Morikawa T. Thoracoscopic resection for intrathoracic neurogenic tumors. Asian J Endosc Surg 2010; **3:** 122-6.

Odaka M, Akiba T, Yabe M, Hiramatsu M, Matsudaira H, Hirano J, Morikawa T. Unilateral thoracoscopic subtotal thymectomy for the treatment of stage I and II thymoma. Eur J Cardiothorac Surg 2010; **37:** 824-6.

Nogi H, Suzuki M, Kamio M, Kato K, Kawase K, Toriumi Y, Takeyama H, Fukushima H, Morikawa T, Uchida K. Impact of CD 44+ CD 24 cells on non-sentinel axillary lymph node metastases in sentinel node-positive breast cancer. *Oncol Rep* 2011; **25:** 1109-15. Epub 2011 Feb 9.

Suzuki T, Akiba T, Miyake R, Marushima H,

Morikawa T. Familial spontaneous pneumothorax in two adult siblings with Marfan syndrome. *Ann Thorac Cardiovasc Surg* 2010; **16:** 362-4.

Suzuki T, Akiba T, Hiramatsu M, Matsudaira H, Hirano J, Odaka M, Morikawa T. Percutaneous cardiopulmonary support of video-assisted metastasectomy for a patient with lung cancer following pneumonectomy. Ann Thorac Cardiovasc Surg 2011; **17**: 45-7.

Morikawa T. Chest surgery (in Japanese). Nippon Rinsho 2010; 68: 1335-9.

Inagaki T, Kiyokawa T, Naganuma H, Hashimoto K, Morikawa T, Hano H. Takayasu arteritis associated with nonspecific interstitial pneumonia and ulcerative colitis. *Jikeikai Med J* 2010; **57:** 33-8.

Reviews and Books

Takeyama H, Takahashi H, Tabei I, Fukuchi O, Nogi H, Kinoshita S, Uchida K, Morikawa T. Malignant neoplasm in the axilla of a male: suspected primary carcinoma of an accessory mammary gland. *Breast Cancer* 2010; **17**: 151-4.

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 the endovascular repair of aneurysms, treatment of peripheral arterial disease with drug-eluting stents, and clinical studies of specific antibodies for heparin-platelet factor 4 (PF4) complexes.

Research Activities

Pediatric Surgery

1. Education

Education for medical students: The patients undergoing pediatric surgery often have congenital anomalies. For this reason, lectures on pediatric surgery for medical students are based on embryology.

Education for physician training : Three objectives for physician training in pediatric surgery are: 1) how to obtain a blood samples from pediatric patients, 2) understanding fluid therapy for pediatric patients, and 3) learning the technique of buried sutures.

Education for surgical residents: They are able to serve as operators or assistants for pediatric surgery.

- 2. Clinical studies
- 1) Manometry for children with constipation
- 2) Testosterone injection for micropenis
- 3) Endoscopic treatment using Deflux, a hyaluronic acid/dextranomer gel (Q-Med AB, Uppsala, Sweden) for grades IV and V vesicoureteral reflux
- 4) Development of introducers for central venous access

5) In cases of severe 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 recovery after surgery is faster. The number of neurologically handicapped children with gastroesophageal reflux has been increasing in our hospital. Carbon oxide changes the balance of electrolytes and acid-base in laparoscopic surgery.

6) The Nuss procedure aims to force the sternum forward and hold it there with an implanted steel bar, but without making 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 The Jikei University Hospital is the third highest in Japan.

7) Molecularly targeted therapy using an antiangiogenic factor

8) Extracorporeal ultrafiltration for septic shock using a rat model

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

9) New diagnostic and therapeutic methods for neuroblastoma

A problem for nursing students is that they have no lectures related to pediatric surgery. A problem for fifth-year medical students is that there is no bedside teaching about pediatric surgery. The curriculum should, therefore, be changed.

Vascular Surgery

1. Development of endovascular repair of thoracoabdominal aneurysms

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

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

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

3. Research on drug-eluting 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 a randomized controlled trial, in which most patients were enrolled in the United States, but also in Germany and Japan, is awaiting its 1-year primary endpoint, which 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 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 the lower extremities becomes longer, and reperfusion syndrome might occur. We have used a small sheath to supply blood flow to the distal lower extremities and to prevent complete ischemia of the lower extremities and consequent reperfusion syndrome.

Publications

Ohki T. EVAR takes root in Japan. *Endovascular Today* 2010; **9:** 46-8.

Ohki T. Cordis incraft system studied in INNO-VATION. *Endovascular Today* 2010; **9:** 14.

Ohki T. Terumo's Misago stent to be studied in US and Japan. *Endovascular Today* 2010; **9**: 22. **Shigematsu H, Nishibe T, Obitsu Y, Matsuzaki K, Ishida A, Miyata T, Shindo S, Hida K, Ohta T, Ando M, Kawasaki T, Yasugi T, Matsumoto T.** Three-year cardiovascular events and disease progress in patients with peripheral arterial disease: results from the Japan Medication Therapy for Peripheral Arterial Disease (J-METHOD). *Int Angiol* 2010; **29(2 Suppl):** 2–13.

Kanaoka Y, Inagaki E, Hamanaka S, Masaki H, Tanemoto K. Analysis of reactive oxygen metabolites (ROMs) after cardiovascular surgery as a marker of oxidative stress. Acta Med Okayama 2010; 64: 323-30.

Enomoto S, Sumi M, Kajimoto K, Nakazawa Y, Takahashi R, Takabayashi C, Asakura T, Sata M. Long-term patency of small-diameter vascular graft made from fibroin, a silk-based biodegradable material. J Vasc Surg 2010; 51: 155-64.
Yamagata T, Yoshizawa J, Ohashi S, Yanaga K, Ohki T. Expression patterns of microRNAs are altered in hypoxic human neuroblastoma cells. Pediatr Surg Int 2010; 26: 1179-84.

Reviews and Books

Lipsitz EC, Veith FJ, Ohki T. Chapter 5 Reducing radiation exposure during endovascular procedure In: Moor WS, Ahn SS, editors. Endovascular surgery 4th ed. Philadelphia: Saunders Elsevier; 2010. p. 41–7.

Kanaoka Y, Ohki T, Veith FJ. Abdominal aortic disease. In: Schillinger M, Minar E, editors. Complex cases in peripheral vascular interventions. London: Informa Healthcare; 2010. p. 131-42.

Sumi M, Ohki T. Chapter 85 Technique: Endo-

vascular therapeutic. In: Cronenwett J, Johnston W, editors. Rutherford's vascular surgery. 7th ed.

Philadelphia: Saunders Elsevier; 2010. p. 1277-94.
Department of Orthopaedic Surgery

Keishi Marumo, Professor Hajime Sugiyama, Associate Professor Hiroki Funasaki, Assistant Professor Makoto Kubota, Assistant Professor Mitsuru Saito, Assistant Professor Hideki Fujii, Assistant Professor Kazuo Asanuma, Associate Professor Takuya Otani, Associate Professor Shigeru Soshi, Assistant Professor Mamoru Yoshida, Assistant Professor Yutaka Ueno, Assistant Professor Iwao Kan, Assistant Professor

General Summary

Basic Research

The research activities of the Department of Orthopaedic Surgery range from experiments on connective tissue cells to clinical studies and have been highly regarded by both Japanese and international orthopaedic societies. This regard is reflected by the participation of department members in the development of treatment guidelines in Japan. Moreover, members of the department continue to win competitive research grants for their ongoing research projects.

Clinical Research

Undertaking clinical research firmly grounded in basic studies and performed in collaboration with many academic and clinical institutions is a well-established and proven standard of high-quality scientific projects carried out at our department. The social and educational imperative of The Jikei University Hospital has been basic and epidemiological studies and the development of new operative methods. The members of our department participate in this assignment, and their efforts have been highly valued.

Research Activities

Arthroscopic surgery for traumatic anterior instability of the shoulder with general joint laxity

The purpose of the present study was to investigate clinical features and results of arthroscopic surgery in 12 patients with traumatic anterior instability of the shoulder complicated by general joint laxity. Eight patients had sustained an initial dislocation owing to minor trauma. Arthroscopic examination showed a poorly developed anterior band of the inferior glenohumeral ligament and medial glenohumeral ligament and a shallow Hill-Sachs lesion in all cases and showed a Bankart lesion with minimal detachment in 9 cases. The mean Japan Shoulder Society Shoulder Instability Score was 55 points before surgery and 84 points after surgery. Postoperative recurrence was observed in 1 patient. The range of motion after surgery was satisfactory in most cases, in which an early recovery was obtained, although the apprehension sign remained, especially in athletes, even 1 year after surgery.

Pathogenesis of the axial symptoms following cervical laminoplasty

Although cervical laminoplasty has been the standard surgical procedure for cervical spondylotic myelopathy, axial symptoms often remain after surgery. We examined the causes of axial symptoms in a prospective controlled study and found that preserving the C7 spinous process could reduce their occurrence.

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

Preoperative and intraoperative patient-specific templating has gained attention as the next technological development after computer-assisted surgery navigation systems in knee surgery. In our department, we have been evaluating the accuracy of implant positioning during total knee arthroplasty with patient-specific bone cutting guides and have been carrying out a prospective study comparing the accuracy of patient-specific templating with that of a 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 establishment of new operation method for flatfoot

We examined results of a new operative method for the treatment of middle-aged patients with flatfoot, in whom the progressive planovalgus deformity developed due to the accessory navicular. In these patients, the connections between the navicular bone and the accessory navicular were extremely loose, and the flatfoot was thought to have developed owing to dysfunction of the tibialis posterior tendon. In 2 cases, in which the accessory navicular was removed and the tibialis posterior tendon reattached, pain remained, and flatfoot did not improve. In 1 case, in which additional lateral column lengthening was adopted, pain decreased comparatively early, and the flatfoot was adequately corrected. We believe that treatment of the accessory navicular is not sufficient and that measures to correct flatfoot should be undertaken to reduce symptoms in such patients.

Femoral reconstruction with a distal interlocking stem following excision of a metastatic tumor in the proximal part of the femur

We examined the effectiveness of a distal interlocking stem for femoral reconstruction in 14 patients who had undergone excision of a metastatic tumor in the proximal part of the femur. The postoperative complications were early death in 1 case and deep tissue infection in another case. In regard to postoperative functional mobility, ambulation was possible in 12 of 13 cases (after excluding a case of early death), and use of a wheelchair was necessary in 1 case. Owing to strengthening of the reconstructed soft tissue, 12 patients were able to elevate their extended leg in the supine position, and 11 patients could elevate their leg in abduction in the lateral position. We conclude that femoral reconstruction with a distal interlocking stem produces satisfactory functional results and is an effective treatment method in patients following excision of the proximal part of the femur due to bone metastasis.

Use of an injectable complex of β -tricalcium phosphate granules, hyaluronate, and fibro-

blast growth factor-2 in the repair of unstable intertrochanteric fractures

We evaluated the effects of an injectable complex of β -tricalcium phosphate (β -TCP) granules, hvaluronate, and recombinant human fibroblast growth factorfactor-22 (rhFGF-2) on the repair of unstable intertrochanteric fractures in elderly patients. Twenty-one patients (age range, 76-91 years) having 31.A2 fractures (AO classification) were treated with an injection of the complex followed by intramedullary nail placement. Fractures with a displaced lesser trochanter resulted in posteromedial cortical defects. Treatment was performed within 8 days after fracture. Bone regeneration and β -TCP resorption, unions of intertrochanteric fractures and displaced lesser trochanters to the shaft, and varus deformity of the femoral neck were assessed with X-ray films and computed tomography scans. Fracture union occurred in all cases, and union of the displaced lesser trochanter to the shaft was obtained in 20 cases by 12 weeks. Interestingly, β -TCP granules were completely replaced by bone, and new bone formation was observed around the lesser trochanter in all cases, unlike cases not treated with the complex. This complex is a paste-like material that is easy to handle and can make a considerable contribution to the treatment of both unstable intertrochanteric fractures and other cortical bone defects with minimal surgical invasion.

Biological markers of bone ageing and the link to osteoporosis: The possible role of bisphosphonates and selective estrogen receptor modulators in the underlying pathophysiology

With aging of the bone collagen fibers, advanced glycation and oxidation end products (AGEs, pentosidine) accumulate in the collagen matrix, and excessive induction of AGEs exacerbates bone fragility. We found that an atherosclerosis risk factor, hyperhomocysteinemia, contributes to the induction of AGE formation. In a study of a Nagano cohort (1,300 cases), we found that high levels of urine AGEs and serum hyperhomocysteinemia may serve as common risk markers of osteoporosis and osteoarthritis.

Publications

Tanaka T, Saito M, Chazono M, Kumagae Y, Kikuchi T, Kitazato S, Marumo K. Effect of alendronate on bone formation and osteoclastic resorption after implantation of beta-tricalcium phosphate. J Biomed Mater Res A 2010; 93: 469-74.

Saito M, Marumo K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. *Osteoporos Int* 2010; **21:** 195-214.

Fukunaga M, Asanuma K, Irie T. Peculiar chondroblastoma involving multiple tarsal bones. *Skeletal Radiol* 2010; **39:** 709-14.

Tanaka S, Yoshimura N, Kuroda T, Hosoi T, Saito M, Shiraki M. The fracture and immobilization score (FRISC) for risk assessment of osteoporotic 2 fracture and immobilization in postmenopausal women. —A joint analysis of the 3 Nagano, Miyama, and Taiji Cohorts—. Bone 2010; 47: 1064-70.

Soshi S, Chazono M, Inoue T, Nakamura Y, Kida Y, Shinohara A, Hashimoto K, Marumo K. Long-term follow up of adolescent idiopathic scoliosis: Evaluation of the outcomes using SRS-22 in surgically treated patients. J Spine Res 2010; **1**: 2101-5.

Ushiku C, Adams DJ, Jiang Xi, Wang L, Rowe DW. Long bone fracture repair in mice harboring GFP reporters for cells within the osteoblastic lineage. *J Orthop Res* 2010; **28**: 1338-47.

Ryu K, Masui F, Saito S, Marumo K. Chronic arthritis of the knee due to synovial metastasis. *Jikeikai Med J* 2010; **57:** 141-7.

Shiraki M, Kuroda T, Shiraki Y, Tanaka S, Higuchi T, Saito M. Urinary pentosidine and plasma homocysteine levels at baseline predict future fractures in osteoporosis under bisphosphonate treatment. J Bone Miner Metab 2011; 29: 62-70.

Jingushi S, Ohfyhu S, Sofue M, Hirota Y, Itoman M, Matsumoto T, Hamada Y, Shindo H, Takatori Y, Yamada H, Yasunaga Y, Ito H, Mori S, Owan I, Fujii G, Ohashi H, Iwamoto Y, Miyanishi K, Iga T, Takahira N, Sugimori T, Sugiyama H. Multiinstitutional epidemiological study regarding osteoarthritis of the hip in Japan. J Orthop Sci 2010: **15**: 626-31.

Tanaka T, Kumagae Y, Marumo K. Openingwedge high tibial osteotomy using a Puddu plate (in Japanese). *Seikei Saigaigeka* 2010; **53:** 819-25.

Tanaka T, Kumagae Y, Marumo K. The knack of opening-wedge high tibial osteotomy using a Puddu plate (in Japanese). *Seikei Saigaigeka* 2010; **53:** 1630-1.

Sugiyama H, Horiuchi D, Nakamura Y. Clinical result of arthroscopic for hip osteoarthritis (in Japanese). *Hip Joint* 2010; **36:** 1-4.

Funasaki H, Yoshida M, Kan I, Kato S, Morohashi M, Kasama K, Marumo K. Arthroscopic Bankart repair for recurrent shoulder dislocation in patients over 40 years of age (in Japanese). *Katakansetsu* 2010; **34:** 355-8.

Yoshida M, Tanaka T, Kumagae Y, Saito M, Suzuki T, Marumo K. Gene expressopn analysis for the synovium of knee in osteoarthritis and rheumatoid arthritis (in Japanese). JOSKAS 2010; **35:** 10-1.

Yoshida M, Funasaki Y, Kan I, Kato S, Kasama K, Marumo K. Gene expression analysis for thick subacromial bursae accompanied with rotator cuff tear (in Japanese). JOSKAS 2011; 36: 22-3.

Chino H, Yukawa M, Maeda K, Marumo K. Treatment of avulsion fracture of the flexor digitorum profundus (in Japanese). *Nihon Te Geka Gakkai Zasshi* 2011; **27**: 787-9.

Chazono M, Soshi S, Inoue T, Kida Y, Nakamura Y, Shinohara A, Marumo K. Relationship of height velocity to skeletal maturity and curve progression in patients with idiopathic scoliosis (in Japanese). *J Spin Res* 2010; **1**: 1936-41.

Chino H, Yukawa M, Maeda K, Okutsu Y, Marumo K. Neuropathy of the deep palmar branch of ulnar nerve with loss of adduction of the index finger and abduction of the middle finger induced by midpalm ganglion; a case report (in Japanese). Seikei Saigaigeka 2011; 54: 309-12.

Fujii H, Bernasek TL, Lyons ST, Otani T, Marumo K. A comparison of post-operative TKA blood loss using epinephrine pain cocktail injection vs platelet rich plasma (in Japanese). Nihon Jinko Kansetsu Gakkaishi 2010; 40: 600-1.

Tamegai H, Otani T, Kawaguchi Y, Fujii H, Ueno Y, Kato T, Ishikawa Y, Marumo K. Lowdose warfarin therapy for prevention of symptomatic pulmonary embolism after hip-surgerypatients who required prolonged bed rest (in Japanese). Nihon Jinko Kansetsu Gakkaishi 2010; 40: 454-5. Tamegai H, Otani T, Kawaguchi Y, Fujii H, Ueno Y, Kato T, Ishikawa Y, Marumo K. Lowdose warfarin therapy for prevention of symptomatic pulmonary embolism after primary total hip arthroplasty (in Japanese). *Hip Joint* 2010; **36**: 503-5.

Kida Y, Saito M, Soshi S, Marumo K. Establishment of non-invasive bone mater quality evaluation method: with indices of skin AGEs reader, blood/urine pentosidine and renal function (in Japanese). *Osteoporosis Japan* 2010; **18:** 639-42.

Umeda M, Kubota M, Yamagishi T, Inoue T, Miyasaka T, Tanaka D, Marumo K. Three cases of peroneal tendon dislocation associated with calcaneal fracture (in Japanese). Kanto Seikei Saigai Geka Gakkai Zasshi 2010; **41:** 76-9.

Maeda K, Chino H, Yukawa M, Okutsu Y, Marumo K. A case report of the reconstruction of distal biceps tendon, one month after the injury (in Japanese). Nihon Te Geka Gakkaishi 2010; 26: 499-500.

Hayama T, Suzuki H, Tino H, Kurosaka D, Ozawa M, Marumo K. Locked knee caused by anomary of plica synovialis; a case report (in Japanese). Seikei Saigaigeka 2010; 53: 993-6.

Nakamura Y, Horiuchi T, Sugiyama H. Arthroscopic mobilization for osteo-arthritis of the hip (in Japanese). *Hip Joint* 2010; **36:** 357-9.

Kinoshita K, Higuchi K, Shimoji D, Higuma M, Saito A, Otani T. Prognosis of socks-handling ability after total hip arthroplasty—for the purpose of effective exercise guidance at leaving hospital— (in Japanese). *Hip Joint* 2010; **36:** 107-9.

Horiuchi T, Nakamura Y, Sugiyama H. Middle-term results of type Y3 total hip system (in Japanese). *Hip Joint* 2010; **36:** 702-4.

Hirano K, Kinoshita K, Otani T. A cadaveric study of functional anatomy of the iliacus muscle (in Japanese). *Hip Joint* 2010; **36:** 189–90.

Reviews and Books

Takahashi N, Maeda K, Ishihara A, Uehara S, Kobayashi Y. Regulatory mechanism of osteoclastogenesis by receptor activator of nuclear factor kB ligand (RANKL) and Wnt signals. *Front Biosci* 2011; **16**: 21-30.

Sugiyama H, Horiuchi T, Nakamura Y. Clinical result and indication of arthroscopic surgery for hip osteoarthritis (in Japanese). *Kansetsugeka* 2010; **29:** 137-44.

Otani T, Kubota M, Kurosaka D, Marumo K. Surgical treatment for the lower limb in rheumatoid arthritis patients (in Japanese). *Nippon Rinsho* 2010; **68 Suppl 5:** 482-5.

Saito M, Marumo K. Bone quality markers: homocysteine and pentosidine (in Japanese). Igaku no Ayumi 2010; 234: 235-6.

Saito M, Marumo K. Arthroscopic debridement (in Japanese). Kansetsu Geka 2010; 29: 1039-43.

Saito M, Marumo K. Bone quality in rheumatoid arthritis (in Japanese). Jin to Kotsutaisha 2010; **23:** 271-80.

Saito M, Marumo K. A change of bony hardness in the osteoporosis (in Japanese). Bio Clinica 2010; 25: 1220-5.

Saito M, Marumo K. Bone strength and bone quality (in Japanese). Rinsho Seikeigeka 2010; 45: 986-93.

Saito M, Marumo K. Estimation of bone quality: the evidence of bone quality markers (in Japanese). *Rinsho Seikeigeka* 2010; **45:** 893-900.

Saito M, Marumo K. Roles of collagen crosslink formation as a determinant of bone quality (in Japanese). *Chiryogaku* 2010; **44:** 16-22.

Saito M. CKD-MBD: Bone quality in chronic kidney disease; enzymatic and non-enzymatic or oxidation induced cross-links in bone (in Japanese). *Clinical Calcium* 2010; **20:** 1068-76.

Saito M. Homocysteine (advanced glycation end products) and bone (in Japanese). *Kotsusoshosho Chiryo* 2010; **9:** 56-63.

Saito M. The reciprocal relation: change of collagen cross-links formation and microdamage in bone mineralization process (in Japanese). *Nihon* Kotsu Keitai Keisoku Gakkaishi 2010; 20: 95-9.

Saito M, Marumo K. Osteoporosis and functional food: vitamin B6, vitamin B12, folic acid (in Japanese). *Functional Food* 2011; **4:** 235-41.

Saito M, Marumo K. Roles of bone mineral density and bone quality as determinants of bone strength (in Japanese). *THE BONE* 2011; **25**: 25-32.

Kida Y, Saito M, Marumo K. How do bisphosphonate, raloxifene, vitamins and PTH affect bone material quality? (in Japanese). *THE BONE* 2010; **24:** 279-87.

Kubota M, Abe M, editors. Kotsu kinniku hifu illustrated: byotaiseiri to assessment (in Japanese). Gekkan Nursing Suppl. Tokyo: Gakken Medical Shujunsha; 2010.

Sugiyama H. Arthroscopic surgery (in Japanese). In: Iwamoto Y, editor. OS NOW Instruction 13. Tokyo: Medical View; 2010. p. 117-27.

Sugiyama H. Arthroscopic surgery (in Japanese). In: Kubo T, Sugiyama H, editors. Osteoarthritis of the hip: Essentials and up-to-date review. Tokyo: Nankodo; 2010. p. 147-52.

Department of Neurosurgery

Toshiaki Abe, Professor Shizuo Oi, Professor Yuichi Murayama, Professor Hisashi Onoue, Associate Professor Yoshiaki Miyazaki, Assistant Professor Yasuko Kusaka, Assistant Professor Toshihiro Ishibashi, Assistant Professor Haruo Sakai, Professor Satoshi Tani, Professor Satoshi Ikeuchi, Associate Professor Yuzuru Hasegawa, Associate Professor Tatsuhiro Joki, Assistant Professor Tosihide Tanaka, Assistant Professor

General Summary

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

Research Activities

Cerebrovascular Disorders

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

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

Endovascular surgery

1. Development of a new endovascular opening system

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

2. Development of a new endovascular operating system

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

3. Development of bioactive coils (Matrix coil)

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

4. Development of Mebiol gel

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

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

5. Flow dynamics for intracerebral aneurysm

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

Brain tumor

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

hand, recent advances in liposome technology have shown promise for the introduction of chemotherapeutic agents with reduced toxicity, extended longevity, and potential for cell-specific targeting. In some previous reports, liposomal doxorubicine was used systemically to treat malignant glioma. In our study we have tried to use doxorubicine and a proteasome inhibitor (MG132) within a thermoreversible polymer for intracranial implantation, a strategy that has been shown to be safe and successful in the treatment of malignant gliomas. We will investigate the release kinetics, toxicity, distribution, and efficacy of this preparation *in vitro* and *in vivo*.

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

Neurotrauma

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

Syringomyelia

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

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

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

2. Fluid in the syrinx

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

Spine and spinal cord group

Numerous conditions, including syringomyelia, degenerative spine diseases, spinal cord tumors, and spinal vascular lesions, have been the major concerns of our depart-

ment. The departments of orthopedic surgery and neurosurgery often collaborate in the interests of patient-orientated treatment in our hospital.

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

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

Pediatric Neurosurgery

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

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

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

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

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

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

Publications

Akasaki Y, Kikuchi T, Irie K, Yamamoto Y, Arai T, Tanaka T, Joki T, Abe T. Cotransfection of Poly (I : C) and siRNA of IL-10 into fusions of den-

dritic and glioma cells enhances antitumor T helper type 1 induction in patients with glioma. *J Immunother* 2011; **34:** 121-8.

Arai T, Benny O, Joki T, Menon LG, Machluf M, Abe T, Carroll RS, Black PM. Novel local drug system using thermoreversible gel in combination with polymeric microspheres or liposomes. Anticancer Res 2010; **30:** 1057-64.

Ishii T, Terao T, Komine K, Abe T. Intramedullary spinal cord metastases of malignant melanoma: an autopsy case report and review of the literature. *Clin Neuropath* 2010; **29:** 334-40. **Takao H, Murayama Y, Ishibashi T, Saguchi T, Ebara M, Arakawa H, Irie K, Iwasaki K, Umezu M, Abe T.** Comparing accuracy of cerebral aneurysm size measurements from three routine investigations: computed tomography, magnetic resonance imaging, and digital subtraction angiography. *Neurol Med Chir* 2010; **50:** 893-9.

Department of Plastic and Reconstructive Surgery

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

General Summary

Research in the Department of Plastic and Reconstructive Surgery is focused on 4 basic areas: 1) the 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.

Evaluation of flap vascularization by intraoperative and postoperative infrared thermal imaging

Success rates of free flap reconstruction of large defects due to excision of malignant tumors are high (no less than 95%). Wound dehiscence and other complications, however, are occasionally seen, especially in patients who have received radiation or chemotherapy or both. Infrared thermography (TVS-200EX, NEC Avio Infrared Technologies, Ltd., Tokyo) is a reliable, noninvasive technique for assessing the vascularization and viability of free flaps and surrounding tissues. It is a useful method for monitoring free flaps and provides valuable information for avoiding complications.

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 acceler-

ates bone formation. We have developed a device with a built-in motor which can produce continuous distraction. Results of experiments using newly developed devices are being investigated.

Tissue engineering

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

Functional analysis of desert hedgehog in patients with macrodactyly

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

Publications

Tanaka S, Yokawa Y, Fujimoto M, Kishi K, Kishi Y. Study of dye laser therapy for strawberry mark (in Japanese). Nihon Laser Igaku Kaishi 2010; **31:** 110-4.

Sakai S, Uchida M. Reconstruction of grip and pinch for symbrachydactyly (in Japanese). Nihon Keisei Geka Gakkai Kaishi 2010; **30:** 341-5.

Ishida K, Kato T, Makino Y, Seino Y, Aoki K, Hirasawa Y, Terao Y, Uchida M. Treatment after total necrosis of free flap reconstruction for head and neck cancer (in Japanese). *Tokeibugan* 2010; **36:** 406-13.

Ninomiya K, Uchida M, Matsuura S. Diagnosis and treatment of periungual tumors (in Japanese). PEPARS 2010; 44: 18-26.

Miyawaki T, Shoji H, Ninomiya K, Matsuura S, Uchida M. Fixation of autogenous bone graft with self-drilling screw in augmentation rhinoplasty (in Japanese). *Nihon Togai Gakuganmen Geka Gakkaishi* 2010; **26:** 284-91.

Department of Cardiovascular Surgery

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General Summary

The main research activities 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. We have examined short-term and mid-term results and confirmed their excellence. Other fields of cardiac surgery were also analyzed and found to have yielded excellent results as well. We are also continuously performing several experimental studies with *in vivo* models. The experimental projects include protection of the heart during cardiac arrest and pulmonary valve function. The major activities are described below.

Research Activities

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

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

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

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

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

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

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

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

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

Clinical studies of pediatric heart surgeries

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

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

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

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

3. The surgical outcome and long-term results of the Ross operation: Effect of autograft dilatation

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

Clinical study of adult cardiac surgery

1. Valve disease

1) Surgical intervention for active mitral infectious endocarditis

Mitral valve repair has been performed even when the leaflets have been severely damaged by active mitral infectious endocarditis. Recently, our basic approach to active mitral infectious endocarditis is to perform surgery early in the active phase while antibiotics are administered, with the aim of improving the rates of cure and of successful mitral valve repair. It is possible to remove vegetations and the infected parts of leaflets and to suture directly or use patch plasty if the infection has not reached the annulus. There are many sources of patch materials which are treated with glutaraldehyde. We are using the Xenomedica patch (Baxter Healthcare Corp., Horw, Switzerland) because of the ease of handling.

2) Mitral valve reconstruction

Leaflet resection for posterior leaflet prolapse has been a standard repair procedure with

good long-term durability. Both quadrangular and triangular leaflet resections tend to cause posterior displacement of the coaptation point, but coaptation line was satisfactory in terms of leaflet geometry. The mobility of the resected leaflet was preserved, and the anterior leaflet could move to coapt with the resected posterior leaflet. Echocardiography revealed excellent mobility of the resected posterior leaflet. Resection procedures have been successfully used to repair mitral valves affected by myxyomatous degeneration.

3) Assessment of our mitral valve repair without systolic anterior motion

We have never encountered systolic anterior motion requiring treatment after mitral valve repair. The key for avoiding systolic anterior motion after mitral valve repair is to resect an excess amount of prolapsed leaflet to obtain a smooth coaptation line, as in single anterior leaflet closure, and to select an appropriately sized ring.

4) Aortic valve replacement

Patient age is an important factor in the choice of valves to replace the aortic valve. The first choice in patients older than 65 years has been a bioprosthetic valve, but such valves are increasingly used for patients younger than 65 years. Long-term results have been satisfactory for both mechanical and bioprosthetic valves. The frequency of bioprosthetic valve use has increased, but only for patients aged 55 to 60 years. In elderly patients the use of mechanical prostheses has been limited to patients with a narrow aortic annulus.

2. Ischemic heart disease

1) Increasing of coronary artery bypass grafting for patients with hemodialysis and the construction of multiple intensive care units

The percentage of patients receiving hemodialysis who undergo coronary artery bypass grafting (CABG) has rapidly increased (2007, 5.1%; 2008, 7.7%; 2009, 12.4%; 2010, 25.0%). The risk of CABG for patients receiving hemodialysis is high, and the postoperative management of these patients may present difficult challenges to the cardiac surgical team. At our institution, specialists from many fields, including nephrology and infection control, participate in the postoperative care of patients in the intensive care unit. This team approach has helped shorten the duration of patients' stays in the intensive care unit, even as the percentage of patients receiving hemodialysis has increased.

2) Training of surgeons in the transitional stage from conventional CABG to off-pump CABG

The procedure of CABG has changed dramatically in the past 10 years from conventional CABG with CPB to CABG without CPB. In our teaching hospital, it would be possible to train resident physicians with the desk-training model system, simple devices, and various anesthetic methods and to achieve satisfactory results.

3. Thoracic aneurysm

1) Preventing cerebrovascular complications in aortic arch replacement

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

Publications

Sakamoto Y, Yoshitake M, Naganuma H, Kawada N, Kinouchi K, Hashimoto K. Reconsideration of patient-prosthesis mismatch definition from the valve indexed effective orifice are. Ann Thorac Surg 2010; 89: 1951-5.

Kawahito K, Adachi H. Balloon catheter pulmonary embolectomy under direct visual control using a choledochoscope. *Ann Thorac Surg* 2011; **91:** 621-3.

Yoshitake M, Takakura H, Sasaki T, Hachiya T, Onoguchi K, Taguchi S, Hoshi T, Hashimoto K. Electron beam cine CT-Based evaluation of left atrial function after the Maze procedure for mitral valve regurtitation. Ann Thorac Cardiovasc Surg 2010; 16: 91–8.

Yamashiro M, Morita K, Uno Y, Shinohara G, Hashimoto K. Modified Norwood procedure with a handmade down-sizing valved right ventricle-to-pulmonary artery conduit. *Gen Thorac Cardiovasc Surg* 2011; **59**: 30-3.

Shinohara G, Morita K, Uno Y, Yamashiro M, Hashimoto K. Scimitar syndrome in an infant with right lung hypoplasia, ventricular septal defect, and severe pulmonary hypertension. *Gen Thorac Cardiovasc Surg* 2010; **58**: 524-7.

Nakamura K, Kawahito K. Erythrocyte-protective effect of sarpogrelate hydrochloride (Anplag®), a selective 5-HT2 receptor antagonist: an in vitro study. *J Artif Organs* 2010; **13**: 178-81.

Nakamura K, Shiratori K, Hashimoto K. Giant saccular aneurysm of coronary arteriovenous fistulato the main pulmonary artery: Intraoperative assessment by using fluorescent imaging. *Ann Thorac Cardiovasc Surg* 2010; **16**: 354-7.

Nakamura K, Kawahito K. Time-related hemolysis in stored shed mediastinal blood after cardiopulmonary bypass. *J Artif Organs* 2011; **14:** 264-7. Epub 2011 Jan 18.

Inagaki T, Kiyokawa T, Naganuma H, Hashimoto K, Morikawa T, Hano H. Takayasu arteritis associated with nonspecific international pneumonia and ulcerative colitis. Jikeikai Med J 2010; 57: 33-8.

Naganuma H, Kawahito K, Matsumura Y, Nakamura K, Haijima N. Octreotide acetate for persistent cylothorax after descending aorta replacement (in Japanese). Kyobugeka 2010; 63: 1124–7.

Miyazaki T, Yamagishi M, Yashima M, Maeda Y, Yamamoto Y, Koide M, Nomura K, Yaku H. Surgical strategy for major aortopulmonary collateral arteries in pulmonary atresia with ventricular septal defect (in Japanese). *Nihon Shoni Junkanki Gakkai Zasshi* 2010; **26**: 392-9.

Department of Obstetrics and Gynecology

Tadao Tanaka, Professor Kazuhiko Ochiai, Professor Takekazu Onda, Professor Seiji Isonishi, Associate Professor Aikou Okamoto, Associate Professor Kuniaki Ohura, Associate Professor Satoshi Takakura, Assistant Professor Kazunori Ochiai, Professor Hiroshi Sasaki, Professor Naoki Kamiya, Professor Shigeki Niimi, Associate Professor Kyousuke Yamada, Associate Professor Hirokuni Takano, Associate Professor

General Summary

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

Research Activities

Gynecologic Oncology

1. Cyclin D1 is a prognostic factor for advanced serous ovarian cancer

We have previously shown with high-resolution oligonucleotide copy number analysis that cyclin E (CCNE1) amplification is strongly associated with the resistance to treatment of serous ovarian cancer. We focused on 66 cases of advanced serous epithelial ovarian cancer (EOC) and investigated the associations between the expression of G1-S phase regulatory proteins and clinicopathological variables. Immunohistochemical analyses of cyclin D1, pRb, p16, p53, p27Kip1, p21Waf1/Cip1, and cyclin E were performed on formalin-fixed tissue sections collected from primary surgical specimens. The correlations between the expression of these proteins and the clinicopathological variables, including progression-free survival (PFS), overall survival (OS), and chemosensitivity, were examined. Univariate analysis showed that overexpression of cyclin D1 was positively correlated with reduced PFS (p=0.00062) and OS (p=0.00037). Reduced expression of p27^{Kip1} tended to be associated with shorter OS (p=0.064). Multivariate analysis showed that overexpression of cyclin D1 (p=0.0019), reduced expression of $p27^{Kip1}$ (p=0.042), and residual tumor volume (p=0.0092) were independent predictors of OS. Overexpression of cyclin D1 (p=0.011) and residual tumor volume (p=0.006) were significantly associated with first-line chemosensitivity. In advanced serous EOC, overexpression of cyclin D1 contributes greatly to poor prognosis owing, perhaps in part, to chemoresistance. Cyclin D1 is a possible target for overcoming the refractory nature of advanced serous EOC.

2. 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 helper T types 1 and 2 cell cytokine response have previously been shown in ovarian cancer. In this study, we sought to clarify whether the cytokine gene expression profile affects the development or progression or both of ovarian cancer.

3. Copy number analysis identifies novel interactions between genomic loci in ovarian cancer

Ovarian cancer is a heterogeneous disease with complex genomic alterations, and, consequently, studies to date have had difficulty determining the most relevant copy number alterations (CNAs). We obtained genome-wide CNA data from 4 different single nucleotide polymorphism array platforms for a final data set of 398 ovarian tumors, mostly of the serous histological subtype. Frequent CNAs targeted many thousands of genes; however, high-level amplicons and homozygous deletions enabled filtering of this list to the most relevant. The large data set enabled refinement of minimal regions and the identification of rare amplicons, such as those at 1p34 and 20g11. We performed a novel co-occurrence analysis to assess the cooperation and exclusivity of CNAs and analyzed their relationships to patient outcome. Positive associations were identified between gains on 19 and 20q, gain of 20q, and loss of X and between several regions of loss, particularly 17q. We found weak correlations of genomic loci, such as 19q12, with clinical outcome. We also assessed genomic instability measures and found a correlation of the number of higher amplitude gains with poorer OS. By assembling the largest collection of ovarian copy number data to date we have been able to identify the most frequent aberrations and their interactions.

4. IL-6-STAT3-HIF signaling and therapeutic response to the angiogenesis inhibitor sunitinib in ovarian clear cell cancer.

Ovarian clear cell adenocarcinoma (OCCA) is an uncommon histotype that is generally refractory to platinum-based chemotherapy. Here, we analyzed the most comprehensive gene expression and copy number data sets compiled to date to identify potential therapeutic targets of OCCA.

Gene expression and DNA copy number analysis were performed with primary human OCCA tumor specimens, and findings were confirmed with immunohistochemical studies of tissue microarrays. Circulating levels of interleukin (IL) 6 were measured in the serum of patients with OCCA or high-grade serous cancers and were related to PFS and OS. Two patients were treated with sunitinib, and their therapeutic responses were measured clinically and with positron emission tomography.

We found specific overexpression of the IL-6-STAT3-HIF (IL-6-signal transducer and activator of transcription 3-hypoxia-induced factor) pathway in OCCA tumors compared with that in high-grade serous cancers. Expression of parathyroid hormone-like hormone and high circulating levels of IL-6 in patients with OCCA may explain the frequent occurrence of hypercalcemia of malignancy and thromboembolic events in OCCA. We describe the amplification of several receptor tyrosine kinases, most notably MET, and suggest other potential therapeutic targets. We report sustained clinical and functional imaging responses in 2 patients with chemotherapy-resistant OCCA who were treated with sunitinib, thus showing significant parallels with renal clear-cell cancer.

Our findings highlight important therapeutic targets in OCCA, suggest that more extensive clinical trials with sunitinib in OCCA are warranted, and provide significant impetus to the growing realization that OCCA is molecularly and clinically distinct from other forms of ovarian cancer.

5. Differential mitochondrial scoring associated with chemotherapeutic responses in patients with ovarian cancer

Ultrathin sections of surgical specimens from patients with ovarian cancer were examined with electron microscopy. Clinical response was compared with mitochondrial scores composed of 7 independent mitochondrial morphological features. The total mitochondrial score was 5.13 in responsive cases and 11.41 in resistant cases. Receiver operating characteristic analysis revealed that the resistant total cut-off score was ≥ 10 points. After a median follow-up period of 20 months, 11 patients have relapsed. The PFS curves showed a difference in favor of the low-scoring patients over high-scoring patients.

6. Clinical and prognostic value of the presence of irregular giant nuclear cells in pT1 ovarian clear cell carcinomas

We aimed to identify irregular giant nuclear cells (IGNCs) with a simple method in clinical practice and to evaluate their prognostic value in pT1 ovarian clear cell carcinomas (OCCCs). Eighty-seven patients with pT1 OCCCs who underwent initial surgery were retrospectively assessed. Survival rates were significantly lower in IGNC-positive patients than in IGNC-negative patients (adjusted hazard ratio=14; 95% confidence interval= 2.7-768). Prognostic differences were not identified for other factors. IGNC identification on 28 available touch imprint cytology smears predicted IGNC identification on paraffin-embedded tissue sections (sensitivity=50.0%, specificity=100%, P=0.007). The presence of IGNCs has clinical and prognostic value for pT1 OCCCs.

Fetomaternal Medicine

1. Does the presence of antiphospholipid antibodies affect the etiology of unexplained infertility as it does that of recurrent spontaneous abortion?

Thrombophilias, such as antiphospholipid antibody (APLA) syndrome, are known to play a role in recurrent spontaneous abortion; however, their effect on unexplained infertility remains controversial and poorly understood. The aim of the present study was to investigate the prevalence of several thrombophilic markers in patients with unexplained infertility so that better use can be made of infertility treatments. In women with unexplained infertility, APLAs of any type tended to have a higher prevalence than in healthy pregnant women; therefore, APLAs might affect reproductive function. We suggest that women with unexplained infertility should undergo screening examinations for APLAs. We intend to investigate more cases.

2. The effect of APLAs on fetal and placental growth

APLAs have been proposed as a possible cause of fetal growth restriction (FGR) related to placental dysfunction. Of the numerous APLAs, however, which are involved is unclear. We revealed the APLA profile implicated in FGR to establish the therapeutic principles for pregnancy in patients with APLAs. In addition, we examined the effects of low-dose aspirin and heparin on the incidence of FGR.

Reproductive endocrinology

1. Study of ending *in vitro* fertilization treatment for infertile women 40 years or older.

Dealing with infertile women 40 years or older who cannot conceive despite *in vitro* fertilization (IVF) treatment can be difficult. The ending of infertility treatment is rarely discussed, although counseling is recognized as being important for infertile patients. We studied the results of IVF treatment in infertile women 40 years or older. We also used a questionnaire survey to examine the ending of IVF treatment for such women.

Publications

Terauchi F, Okamoto A, Wada Y, Hasegawa E, Sasaki T, Akutagawa O, Sagawa Y, Nishi H, Isaka K. Incidental events of diaphragmatic surgery in 82 patients with advanced ovarian, primary peritoneal and fallopian tubal cancer. Oncol Lett 2010: **1**: 861–4.

Gorringe KL, George J, Anglesio MS, Ramakrishna M, Etemadmoghadam D, Cowin P, Sridhar A, Williams LH, Boyle SE, Yanaihara N, Okamoto A, Urashima M, Smyth GK, Campbell IG, Bowtell DD; Australian Ovarian Cancer Study. Copy number analysis identifies novel interactions between genomic loci in ovarian cancer. *PLoS One* 2010; **5**: e11408.

Anglesio MS, George J, Kulbe H, Friedlander ML, Rischin D, Lemech C, Power J, Coward J, Cowin PA, House CM, Chakravarty P, Gorringe KL, Campbell IG; Australian Ovarian Cancer Study Group, Okamoto A, Birrer MJ, Huntsman DG, de Fazio A, Kalloger SE, Balkwill F, Gilks B, Bowtell DD. ILG-STAT3-HIF signalling and therapeutic response to the angiogenesis inhibitor, sunitinib, in ovarian clear cell cancer. *Clin Cancer Res* 2011; **17**: 2538-48. Epub 2011 Feb 22.

Hashimoto T, Yanaihara N, Okamoto A, Nikaido T, Saito M, Takakura S, Yasuda M, Sasaki H, Ochiai K, Tanaka T. Cyclin D1 predicts the prognosis of advanced serous ovarian cancer. Exp Ther Med 2011; 2: 213-9.

Matsumoto R, Isonishi S, Ochiai K, Hamada T, Kiyokawa T, Tachibana T, Ishikawa H. Prognostic significance of mitochondrial scoring system in ovarian cancer. Exp Ther Med 2010; 1: 783-8. Isonishi S, Suzuki M, Hirama M, Matsumoto R, Ochiai K, Tanaka T. Use of docetaxel after paclitaxel hypersensitivity reaction in epithelial ovarian and endometrial cancer. Clin Ovarian Cancer 2010: 2: 44-7.

Anglesio MS, Carey MS, Köbel M, Mackay H, Huntsman DG; Vancouver Ovarian Clear Cell Symposium Speakers. Clear cell carcinoma of the ovary: a report from the first Ovarian Clear Cell Symposium, June 24th, 2010. *Gynecol Oncol* 2011; **121:** 407-15. Epub 2011 Jan 26.

Yanaihara N, Okamoto A, Yanagida S, Ochiai K, Tanaka T. Molecular genetic of gynecological cancer (in Japanese). *Nippon Rinsho* 2010; **68** Suppl 8: 489-93.

Nakajima K, Isonishi S, Saito M, Tachibana T, Ishikawa H. Characterization of two independent, exposure-time dependent paclitaxel-resistant human ovarian carcinoma cell lines. *Human Cell* 2010; 23: 156-63.

Department of Urology

Shin Egawa, Professor Isaho Ikemoto, Associate Professor Hiroshi Kiyota, Associate Professor Nozomu Furuta, Assistant Professor Takashi Hatano, Assistant Professor Shoichi Onodera, Professor Koichi Kishimoto, Associate Professor Koji Asano, Associate Professor Yasuyuki Suzuki, Assistant Professor Kenta Miki, Assistant Professor

General Summary

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

Research Activities

Urologic oncology

1. Basic research: We have several projects that aim to clarify the biology of urological malignancies and to develop new therapeutic tools. Most projects were reported at the annual meeting of the Japanese Urological Association and the American Urological Association. The projects are as follows.

1) Proteomic analysis of new biomarkers for prostate cancer and urothelial cancer

2) Establishment and biological analysis of a new prostate cancer model derived from Japanese patients

3) Research on neurourology and female urology

2. Clinical research: Several clinical studies are ongoing in our department. 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 against high-risk prostate cancer

- 3) Study of deep venous thrombosis after urological surgery
- 4) Study of the incidence of latent prostate cancer
- 5) Clinical study of cryosurgery for small renal tumors

6) Clinical study of neoadjuvant/presurgical treatment with molecularly targeted therapy for renal cell carcinomacarcinoma

Publications

Hatano T, Uno T, Tsuduki S, Koike Y, Hata K, Kishimoto K, Mogami T, Sunakawa Y, Harada J, Egawa S. Chronological changes after MRIguided percutaneous cryotherapy for small renal tumors. *Teion Igaku* 2010; **36:** 103-8.

Miki K, Kiba T, Sasaki H, Kido M, Aoki M, Takahashi H, Miyakoda K, Dokiya T, Yamanaka H, Fukushima M, Egawa S. Transperineal prostate brachytherapy, using I-125 seed with or without adjuvant androgen deprivation, in patients with intermediate-risk prostate cancer: study protocol for a phase III, multicenter, randomized, controlled trial. *BMC Cancer* 2010; **10**: 572. Matsumoto Y, Miyazato M, Furuta A, Torimoto

K, *Hirao Y*, *Chancellor MB*, *Yoshimura N*. Differential roles of M2 and M3 muscarinic receptor subtypes in modulation of bladder afferent activity in rats. *Urology* 2010; **75**: 862-7.

Nickel JC, Furuta A, Chancellor MB, Roehrborn CG, Assimos DG, Shapiro E, Brawer MK. Best of the AUA annual meeting: highlights from the 2010 American Urological Association meeting, May 29-June 3, 2010, San Francisco, CA. *Rev Urol* 2010; **12:** e134-46.

Kimura T, Hiraoka K, Kasahara N, Logg CR. Optimization of enzyme-substrate pairing for bioluminescence imaging of gene transfer using Renilla and Gaussia lucigerases. *J Gene Med* 2010; **12:** 528-37.

Kyosen SO, lizuka S, Kobayashi H, Kimura T, Fukuda T, Shen J, Shimada Y, Ida H, Eto Y, Ohashi T. Neonatal gene transfer using lentiviral vector for murine Pompe disease: long-term expression and glycogen reduction. *Gene Ther* 2010; **17:** 521-30.

Kubo S^{1, 2}, Kataoka M³, Tateno C³, Yoshizato K^{3, 4}, Kawasaki Y², Kimura T¹, Faure-Kumar E¹, Palmer DJ⁵, Ng P⁵, Okamura H², Kasahara N¹ ('UCLA, ²Hyogo Coll Med, ³Hiroshima Pref Inst Ind Sci Tech, ⁴Hiroshima Univ, ⁵Baylor Coll Med). In vivo stable transduction of humanized liver tissue in chimeric mice via high-capacity adenovirus- lentivirus hybrid vector. Human Gene Ther 2010; **21**: 40-50.

Sasaki H, Miki J, Kimura T, Yamamoto T, Koike Y, Miki K, Egawa S. Upfront transection and subsequent ligation of the dorsal vein complex during laparoscopic radical prostatectomy. Int J Urol 2010; 17: 960-1.

Department of Ophthalmology

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

General Summary

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

Research Activities

Cataract

The widespread use of ultrasound technology in cataract surgery and the introduction of foldable intraocular lenses (IOLs) have allowed cataract surgery and IOL implantation through incisions of 2.4 to 3.0 mm. Surgeons are now experimenting with even smaller incisions. We began to use a standard phacoemulsification and aspiration (PEA) device to perform bimanual PEA with a sleeveless phaco tip through incisions 1.2 to 1.4 mm wide. We used an irrigating hook through a side port to infuse the anterior chamber. After the lens was extracted, we were able to implant safely hydrophobic acrylic single-piece IOLs through a 1.8-mm incision. We can 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. Neuromyelitis optica (NMO) is characterized by optic neuritis (ON) and acute myelitis. In 2004, an antibody specific for NMO was discovered. This antigen turned out to be aquaporin-4 (AQP4), a water channel predominantly expressed within the central nervous system. We described a patient with NMO in whom anti-AQP4 antibodies were present and myelitis developed 25 years after the onset of ON. This case fulfilled all 3 defining criteria for NMO. The present case also showed the longest documented period to date between the onset of ON and the subsequent development of myelitis. The length of the interval between these events may be irrelevant to the diagnostic criteria for NMO. When a patient who has ON and anti-AQP4 antibodies exhibits numbness and paralysis over the long term, ophthalmologists and neuro-ophthalmologists should recognize the possibility of myelitis, even when the patient has no history of myelitis.

2. We started a randomized, parallel-group, multicenter clinical trial of treatment with topical unoprostone for acute nonarteritic anterior ischemic optic neuropathy. Criteria for eligibility included an age of 50 years or older, duration of symptoms less than 2 weeks, visual loss or visual field defects consistent with optic neuropathy, absence of pain, a relative afferent pupillary defect, and optic disc edema. The primary endpoint was the mean deviation on perimetry with the Humphrey field analyzer at 12 months.

3. In patients who had undergone temporal lobectomy for epilepsy, we assessed the correlation between degeneration along Meyer's loop and postoperative visual field defects by means of diffusion tensor imaging, which can be used to evaluate axonal integrity in the white matter. The probabilistic fiber tracking method demonstrated axonal disruption over the temporal horn of the lateral ventricle. Diffusion tensor imaging would be a useful method for delineating Meyer's loop.

4. We reported two cases of recurrent ON with panhypopituitarism caused by hypophysitis, anti-aquaporin 4 antibody-positive familial neuromyelitis optica in a mother and daughter, and an atypical case of papilledema in a patient with a jugular foramen tumor. These reports described etiologic relationships and were highly suggestive.

Ocular oncology and histopathology

1. Mixed tumors originating from both tubular epithelial cells and myoepithelial cells usually develop in the major lacrimal gland of the ocular adnexa. They rarely develop in the eyelid. Mixed tumors arising from Moll's glands are extremely rare. We reported a rare mixed tumor of Moll's gland origin in the margin of the lower eyelid. Histopathological examination showed a well-circumscribed nodular lesion in the dermis containing fibrous, hyaline, and myxoid elements. The tumor showed cords and nests of proliferating tubular epithelial cells and myoepithelial cells. Spindle-shaped myoepithelial cells were observed. The tumor was diagnosed as a mixed tumor. On immunohistochemical analysis the tumor cells were positive for S-100 protein, and proliferative lesions of tubular epithelial cell origin were strongly positive for gross cystic disease fluid protein-15 (GCDFP-15). Mixed tumors of the eyelid may arise from the accessory lacrimal glands of Krause and Wolfring, eccrine sweat glands, or apocrine (Moll's) sweat glands. This tumor was considered to be of Moll's gland origin because of its location, the features of the surrounding tissue, its composition of tubular epithelial cells, and its positive staining for GCDFP-15.

2. We reported rare cases of malignant solitary fibrous tumors of the eyelid in a patient with myotonic dystrophy, giant subretinal hematoma improved by vitreous hemorrhage with presumed vasoproliferative retinal tumor, and spontaneous regression of hemangioma in the optic disc. These reports described the etiologic relationships and were highly suggestive.

Glaucoma

1. We evaluated the validity of the visual field index slope (VFIS) compared with the pattern standard deviation slope (PSDS) for assessing glaucomatous progression. In a large percentage of eyes examined, the VFIS and PSDS did not agree, and the results sug-

gest that VFIS may be more affected by central weighting of the index than is traditional PSDS.

2. We evaluated the effect of intraocular pressure (IOP) reduction and the safety of 0.0015% tafluprost ophthalmic solution in patients with normal-tension glaucoma with an IOP of 16 mm Hg or less. Tafluprost significantly reduced IOP without apparent safety concerns in these patients.

3. We compared the change in wavefront aberrations after simultaneous cataract surgery and trabeculectomy through the same wound under the scleral flap and through a separately created corneal incision. Wavefront aberration analysis was an effective method for evaluating ocular function after the operation, and the results suggest that differences in the incisions for combined surgery affect postoperative aberrations.

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 images. Both types of images were acquired with a clinical MR scanner. Fifteen patients with glaucoma and 15 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 to the visual pathway occur in intracranial structures as well as in the eyes of patients with glaucoma.

Developmental functional abnormality

Binocular summation on the visual cortex was explored with fMRI in patients with postoperative strabismus and in healthy volunteers. Binocular summation was demonstrated to be less important for patients with strabismus at the foveal projection area and at a peripheral 2-degree projection area. This result suggests that abnormal cortical visual processing is present as cortical suppression of the prefoveal projection area in patients with strabismus.

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 adult human V1

We extended the measurement to subject with retinitis pigmentosa. Our results were same as in patients with macular degeneration; there is no large-scale remapping in the adult human V1. Our results support vision-restoring therapies that rely on the stability of the human V1.

3. Three papers published in international journals

Three of our studies previously reported here have been published: objective perimetry using 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 removing 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 membrane, macular edema, and rhegmatogenous retinal detachment. The 25- and 23-gauge sutureless vitrectomy techniques decrease surgical trauma and improve patients' postoperative comfort. The 25- and 23-gauge instrumentation is effective for a variety of vitreoretinal surgical indications. Although the infusion and aspiration rates of the 25- and 23-gauge instruments are lower than those of the 20-gauge high-speed vitrectomy system, the use of 25- and 23-gauge transconjunctival vitrectomy systems may effectively reduce operative times for selected cases that do not require the full capability of conventional vitrectomy.

To evaluate the clinical efficacy of 7-mm IOLs (Eternity IOL, Santen Pharmaceutical Co., Ltd., Osaka) for combined pars plana vitrectomy, phacoemulsification, and IOL implantation, we examined 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 whether functional disorders are present in retinal cell levels in 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 examinations with 4 types of recording system: 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. Furthermore, the results (objective examination) using the multifocal stimulator can be compared with those of the visual field tests (subjective examination). The color ERG records each response to separate long-, middle-, 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 analyze them 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. 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 retinal function by recording ERGs in patients with diabetes in whom retinopathy is absent on ophthalmoscopy. We measured photopic negative responses (PhNRs) among wave patterns obtained in cone ERGs and examined the correlation between PhNRs and the duration of diabetes. We are measuring the thickness of the nerve fiber layer with optical coherence tomography and are investigating the correlation of nerve fiber layer thickness with PhNR amplitude and implicit time.

Uveitis

1. A novel therapy with a chimeric antibody against tumor necrois 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. Cyclophosphamide pulse therapy for antineutrophil cytoplasmic antibody-associated scleritis

Antineutrophil cytoplasmic antibody (ANCA)-associated active scleritis tends to have more severe inflammation and requires more aggressive therapy. We reported a case of necrotizing scleritis positive for both cytoplasmic pattern ANCA and perinuclear staining pattern ANCA without underlying systemic vasculitis which was successfully treated with intravenous pulse cyclophosphamide therapy.

3. Cyclophosphamide pulse therapy for severe systemic lupus erythematosus retinopathy.

Any form of systemic lupus erythematosus retinopathy is more likely to be aggressive. We reported a case of severe systemic lupus erythematosus retinopathy that was successfully treated with intravenous pulse cyclophosphamide therapy.

Macular degeneration

1. Photodynamic therapy combined with intravitreal ranibizumab for neovascular agerelated macular degeneration in Japanese patients.

We examined 3-month results of photodynamic therapy (PDT) with verteporfin combined with intravitreal ranibizumab for neovascular age-related macular degeneration (AMD). After 3 months PDT combined with intravitreal ranibizumab appeared to be effective for Japanese patients with neovascular AMD.

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

We evaluated the efficacy of triple therapy consisting of a single session of PDT, intravitreal bevacizumab, and subtenon triamcinolone acetonide as the initial therapy for polypoidal choroidal vasculopathy (PCV). The present study showed that initial therapy consisting of a single session of PDT, intravitreal bevacizumab, and subtenon triamcinolone acetonide improves vision and reduces central macular thickness in PCV.

3. We reported a case delayed radiation maculopathy and papillopathy treated with intravitreal bevacizumab after irradiation for maxillary sinus cancer.

4. Cataract surgery and typical AMD

We compared background factors of the 2 predominant subtypes of exudative AMD in the Japanese population: typical AMD and PCV. A history of cataract surgery and a history of central serous chorioretinopathy are more frequent in typical AMD and in PCV, respectively.

Biochemistry

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

2. Inhibition of extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase suppresses interleukin (IL)-23- and IL-1-driven IL-17 production and attenuates autoimmune disease. We investigated the effects of an ERK inhibitor (PD98059 or U0126) on EAU. The ERK inhibitor exhibited significant anti-inflammatory and immunosuppressive effects in EAU. The ERK inhibitor is a promising therapeutic modality for autoimmune uveitis.

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 diagnosed with 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 peak absorbance 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 peak absorbance by 4 nm and 8 nm, respectively. Our results suggest that the genotype of a female proband with deuteranomaly can be determined when both genetic and color vision testing are performed in 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 controls.

3. We investigated the involvement of various genetic factors in Japanese patients with AMD, which is a common cause of blindness in elderly persons in industrialized countries. More than 500,5688 single nucleotide polymorphisms of the whole genome were genotyped with Affymetrix Human Mapping Arrays (Affymetrix, Santa Clara, CA, USA) and the TaqMan assay (Applied Biosystems, Inc., Carlsbad, CA, USA). We are now analyzing candidate single nucleotide polymorphisms involved in Japanese patients with AMD.

Cornea

The cornea group at The Jikei University selects the most-appropriate corneal surgery by discussing the various options with each patient. Automated lamellar therapeutic keratectomy, in which a microkeratome is used to make a lamellar flap, was performed in several cases of 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.

Publications

Istoc A, Habas C, Iba-Zizen MT, Nguyen TH, Abanou A, Yoshida M, Bellinger L, Le Gargasson JF, Cabanis EA. Value of the functional neural tractography in the reconstruction of the visual pathways in DTMRI (in French). J Fr Ophtalmol 2010; **33:** 670-9.

Watanabe A, Gekka T, Shibata T, Takashina H, Tsuneoka H. Twenty-three-gauge vitrectomy with bevacizumab in patients having proliferative diabetic retinopathy with an active fibrovascular membrane. Jikeikai Med J 2010; 57: 67-71.

Watanabe A, Shibata T, Ozaki M, Okano K, Kouzaki K, Tsuneoka T. Change in anterior chamber depth following combined pars plana vitrectomy, phacoemulsification, and intraocular lens implantation using different types of intraocular lenses. Jpn J Ophthalmol 2010; 54: 383-6.

Sakai T, Kanetaka A, Noro T, Tsuneoka H. Intraocular surgery in patients receiving infliximab therapy for Behçet disease. Jpn J Ophthalmol 2010; 54: 360–1.

Sakai T, Ishihara T, Higaki M, Akiyama G, Tsuneoka H. Therapeutic effect of stealth-type polymeric nanoparticles with encapsulated betamethasone phosphate on experimental autoimmune uveoretinitis. Invest Ophthalmol Vis Sci 2011; **52:** 1516-21.

Hayashi T, Tsuzuranuki S, Kozaki K, Urashima M, Tsuneoka H. Macular dysfunction in Oguchi disease with the frequent mutation 1147delA in the SAG gene. Ophthalmic Res 2011; **46:** 175-80.

Rocha Sousa A, Hayashi T, Gomes NL, Penas S, Brandão E, Rocha P, Urashima M, Yamada H, Tsuneoka H, Falcão Reis F. A novel mutation (Cys83Tyr) in the second zinc finger of NR2E3 in enhanced S-cone syndrome. Graefes Arch Clin Exp Ophthalmol 2011; 249: 201-8.

Masuda Y, Horiguchi H, Dumoulin SO, Furuta A, Miyauchi S, Nakadomari S, Wandell BA. Task-dependent V1 responses in human retinitis pigmentosa. Invest Ophthalmol Vis Sci 2010; 51: 5356-64.

Ogasawara M, Shikishima K, Sakai T, Takagi M, Tanaka K. A case of neuromyelitis optica developing into myelitis 25 years after optic neuritis. Jpn J Ophthalmol 2010; **54**: 372-3.

Winawer J, Horiguchi H, Sayres RA, Amano K, Wandell BA. Mapping hV4 and ventral occipital cortex: the venous eclipse. J Vis 2010; 10: 1. Maeda A, Okano K, Park PS, Lem J, Crouch **RK, Maeda T, Palczewski K.** Palmitoylation stabilizes unliganded rod opsin. *Proc Natl Acad Sci U S A* 2010; **107:** 8428-33.

Sakami S, Maeda T, Bereta G, Okano K, Golczak M, Sumaroka A, Roman AJ, Cideciyan AV, Jacobson SG, Palczewski K. Probing mechanisms of photoreceptor degeneration in a new mouse model of the common form of autosomal dominant retinitis pigmentosa due to P23H opsin mutations. J Biol Chem 2011; **286:** 10551-67.

Department of Otorhinolaryngology

Hiroshi Moriyama, Professor Tohru Imai, Associate Professor Hiromi Kojima, Associate Professor Makoto Iida, Assistant Professor Mamoru Yoshikawa, 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, cases of cholesteatoma surgery performed at our hospital are recorded in our database, which is used to analyze the condition of patients, to select operative methods, and to review postoperative outcomes. In regard to research on hearing loss, we are studying the physiology of the inner ear in metabolic disorders using experimental animal models and collaborating with Shinshu University in the genetic analysis of deaf patients.

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

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

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

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

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

Research in rhinology

We are involved in the analysis of data on factors related to the intractability of rhinosinusitis obtained from patients undergoing endoscopic sinus surgery (ESS) and from prospective studies of the postoperative course. In an attempt to expand the indications for ESS from paranasal sinus tumors to skull-base surgery, including that for spinal fluid leakage, skull-base tumors, and pituitary gland tumors, and to improve the safety of ESS, we have performed high-tech navigation surgery in which 3-dimensional endoscopic images and stereonavigation images are superimposed. We have identified problems and possible areas of improvement relevant to this operative method and are altering the device to improve its accuracy and performance.

This year, we began developing clinical studies and treatment methods for patients with a variety of olfactory disorders. Since last year we have offered anatomy training using fresh-frozen cadavers at skills laboratory, and went to skull-base surgery and ordinary endoscopic sinus surgery training. We reflected improvements in procedures under the endoscopic skull-base surgery and paranasal sinus tumorectomy in result. In addition, we started creating new methods of Internet access using telemedicine and a distance-training system.

Research issues on head and neck tumors

For common advanced cancers we perform radical surgery (e.g., total pharyngolaryngectomy combined with reconstruction by means of free intestinal flap transfer for hypopharyngeal cancer and total laryngectomy for laryngeal cancer); however, we perform larynxpreserving surgery (partial hypopharyngectomy combined with reconstruction by means of free-flap transfer and partial laryngectomy) to preserve function, especially vocal function, to the greatest extent possible. We have obtained favorable outcomes in terms of both laryngeal preservation and survival. For conservative therapy and postoperative treatment for advanced cancer, we perform radiotherapy, alone or with concurrent chemotherapy with cisplatin and 5-fluorouracil, and have obtained favorable results. We use narrow-band imaging endoscopy for diagnosis in routine practice and make good use of this technology for the diagnosis and treatment of early-stage superficial mesopharyngeal and hypopharyngeal cancers.

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

Research on vocal and swallowing functions

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

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

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

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

Research on sleep apnea syndrome

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

Continuous positive airway pressure treatment will be the first choice for patients with obstructive sleep apnea syndrome of greater than moderate severity. On the other hand, the effectiveness and safety questioned surgical treatment. Therefore, we analyzed about the adaption of surgical treatment for sleep disorders such an uvulopalatopharyngoplasty representatively.

Long-distance sleep examinations have been performed since 2009 at the Ota Sleep Science Center.

Publications

Wada K, Matsuwaki Y, Yoon J, Benson LM, Checkel JL, Bingemann TA, Kita H. Inflammatory responses of human eosinophils to cockroach are mediated through protease-dependent pathways. J Allergy Clin Immunol 2010; 126: 169-72, e2.

Wong WK, Matsuwaki Y, Omura K, Moriyama H. Role of intraoperative CT-updates during image-guided endoscopic sinus surgery for sinonasal fibro-osseous lesions. *Auris Nasus Larynx* 2011; **38:** 628-31. Epub 2011 Jan 11.

Okushi T, Tonogi M, Arisaka T, Kobayashi S, Tsukamoto Y, Morishita H, Sato K, Sano C, Chiba S, Yamane GY, Nakajima T. Effect of maxillomandibular advancement on morphology of velopharyngeal space. J Oral Maxillofac Surg 2011; 69: 877-84.

Sakurai Y, Suzuki R, Yoshida R, Kojima H, Watanabe M, Manome Y, Ohashi T, Eto Y, Moriyama H. Inner ear pathology of alphagalactosidase A deficient mice, a model of Fabry disease. Auris Nasus Larynx 2010; **37**: 274-80. **Tahara M, Araki K, Okano S, Kiyota N, Fuse N, Minashi K, Yoshino T, Doi T, Zenda S, Kawashima M, Ogino T, Hayashi R, Minami H, Ohtsu A.** Phase I trial of combination chemotherapy with docetaxel, cisplatin and S-1 (TPS) in patients with locally advanced or recurrent/metastatic head and neck cancer. Ann Oncol 2011; **22**: 175-80.

Sano H, Matsuwaki Y, Kaito N, Joki T, Okushi T, Moriyama H. A case of sphenoid sinus meningoencephalocele repaired by an imageguided endoscopic endonasal approach. *Auris Nasus Larynx* 2011; **38:** 632-7. Epub 2011 Mar 9.

Reviews and Books

Matsuwaki Y, Moriyama H. Progress in endoscopic sinus surgery (in Japanese). *Nippon Rinsho* 2010; **68:** 1360-5.

Department of Anesthesiology

Shoichi Uezono, Professor Sachiko Omi, Professor Masanori Takinami, AssociateProfessor Ichiro Kondo, Associate Professor Shigehiko Uchino, Associate Professor Yoshie Taniguchi, Assistant Professor Kazuhiro Shoji, Assistant Professor Hiroshi Sunaga, Assistant Professor Takehiko Nezu, Professor Shuya Kiyama, Associate Professor Masaki Kitahara, Associate Professor Yasushi Mio, Associate Professor Masamitsu Sanui, Associate Professor Chieko Fujiwara, Assistant Professor Yoichi Kase, Assistant Professor

General Summary

The 2010 academic year is the sixth year that the Department of Anesthesiology has been directed by Chairman Professor Shoichi Uezono, M.D. The functions of the Department of Anesthesiology are to provide quality patient care, to teach, and to perform research in perioperative medicine, intensive care medicine, and comprehensive pain management. In 2010 we made further advances and great achievements with the support of our faculty, institutional administration, and the dean of The Jikei University. Below we highlight some of our research achievements in 2010.

Research Activities

Research continues as a growing and important component of the department's activities. The department is committed to enhancing academic productivity and resources by dedicating time to research and granting clinical access to research cases. The investigators have been successful each year in obtaining peer-reviewed research grants, such as Grants-in-Aid for Scientific Research (Kakenhi) and contract grants. The department continues to build on the strengths of several outstanding programs: cardiovascular anesthesia, thoracic anesthesia, pediatric anesthesia, regional anesthesia, neuroanesthesia, obstetric anesthesia, intensive care medicine, and comprehensive pain management. Faculty recruitment is targeted at individuals with demonstrated academic and research productivity as well as excellent clinical management and teaching skills. In 2010, the department was able to invite Dr. Sanui from Jichi Medical College as an associate professor. Dr. Sanui is a nationally and internationally recognized expert in intensive care medicine and an excellent teacher of perioperative critical care. Dr. Sunaga successfully finished his 2-year research fellowship at Cornell University in the United States and re-joined our faculty. The Japanese Society of Anesthesiologists' annual meeting in Fukuoka and the American Society of Anesthesiologists' annual meeting in San Diego were both well represented by our faculty and residents.

Highlighted below are the ongoing research projects in which the principal investigators were faculty members of the Department of Anesthesiology.

Dr. Uezono's research focus has been pulmonary vascular physiology and its clinical application to children with single ventricular physiology. Dr. Omi is interested in the
development of simulation tools to learn how to deliver peripheral nerve blocks. Dr. Taniguchi has been interested in temperature regulation during surgery and its effects on postoperative outcomes in patients undergoing head and neck surgery. Dr. Kiyama examined the effects of propofol on the bispectral index in elderly patients and determined the optimal dose of propofol in this population. Members of the intensive care medicine staff (Drs. Takinami, Sanui, Uchino, and Kase) have remained extremely active in clinical research on the following topics: (1) the usefulness of criteria for acute disseminated intravascular coagulation to predict outcomes in patients admitted to the intensive care unit, (2) blood levels of vitamin D and genetic polymorphism of vitamin D receptors in patients after cardiac surgery, and (3) the efficacy of sivelestat in sepsis-induced acute lung injury. Dr. Kitahara and his colleagues in the Pain Clinic continue their efforts to establish standards of objective pain assessment. He is also assessing the effects of tramadol on various types of chronic pain. Dr. Kojima has been an active member of a 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 effects of long-term administration of morphine on granuloma formation in the spinal cord (Dr. Kondo), mechanisms of anesthetic post-conditioning in myocardial mitochondria (Dr. Mio), and the effects of donepezil on the pharmacokinetics of neuromuscular blocking agents (Dr. Sunaga). A total of 5 Grants-in-Aid for Scientific Research (*Kakenhi*) were obtained in 2010.

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

Publications

Sunaga H, Zhang Y¹, Savarese JJ², Emala CW¹ (¹Coll Phys Surg Columbia Univ, ²Weill Cornell Med Coll). Gantacurium and CW 002 do not potentiate muscarinic receptor-mediated airway smooth muscle constriction in guinea pigs. Anesthesiology 2010; **112:** 892-9.

Sunaga H, Malhotra JK¹, **Yoon E**¹, **Savarese JJ**¹, **Heerdt PM**¹ (**'Weill Cornell Med Coll)**. Cysteine reversal of the novel neuromuscular blocking drug CW002 in dogs: Pharmacodynamics, acute cardiovascular effects, and preliminary toxicology. *Anesthesiology* 2010; **112**: 900-9.

Iwai K, Uchino S, Endo A, Saito K, Kase Y, Takinami M. Prospective external validation of the new scoring system for disseminated intravascular coagulation by Japanese Association for Acute Medicine (JAAM). *Thromb Res* 2010; **126**: 217-21.

Terui T, Shimamoto Y, Yamane M, Kobirumaki F, Ohtsuki I, Ishiwata S, Kurihara S, Fukuda N. Regulatory mechanism of length-dependent activation in skinned porcine ventricular muscle: role of thin filament cooperative activation in the Frank-Starling relation. *J Gen Physiol* 2010; **136**: 469–82.

Uchino S, Bellomo R, Bagshaw SM, Gold-

smith D. Transient azotaemia is associated with a high risk of death in hospitalized patients. *Nephrol Dial Transplant* 2010; **25**: 1833-9.

Heerdt PM¹, Malhotra JK¹, Pan BY¹, Sunaga H, Savarese JJ¹ ('Weill Cornell Med Coll). Pharmacodynamics and cardiopulmonary side effects of CW 002, a cysteine-reversible neuromuscular blocking drug in dogs. *Anesthesiology* 2010; **112**: 910-6.

Savarese JJ¹, McGilvra JD², Sunaga H, Belmont MR¹, Van Ornum SG², Savard PM¹, Heerdt PM¹ ('Weill Cornell Med Coll, ²Cedarburg Phar). Rapid chemical antagonism of neuromuscular blockade by L-cysteine adduction to and inactivation of the olefinic (double bonded) isoquinolinium diester compounds gantacurium (AV 430A), CW 002, and CW 011. Anesthesiology 2010; **113**: 58–73.

Srisawat N, Lawsin L, Uchino S, Bellomo R, Kellum JA; BEST Kidney Investigators. Cost of acute renal replacement therapy in the intensive care unit: results from The Beginning and Ending Supportive Therapy for the Kidney (BEST Kidney) study. Crit Care 2010; 14: R46.

Sedlic F, Pravdic D, Hirata N, Mio Y, Sepac A, Camara AK, Wakatsuki T, Bosnjak ZJ, Bienengraeber M. Monitoring mitochondrial electron fluxes using NAD(P)H-flavoprotein fluorometry reveals complex action of isoflurane on cardiomvocvtes. Biochim Biophys Acta 2010: **1797:** 1749-58.

Yamada M, Kida K, Amutuhaire W, Ichinose F, Kaneki M. Gene disruption of caspase-3 prevents MPTP-induced Parkinson's disease in mice. Biochem Biophys Res Commun 2010; 402: 312-8.

Bougaki M, Searles RJ, Kida K, Yu J, Buys ES. Ichinose F. Nos3 protects against systemic inflammation and myocardial dysfunction in murine polymicrobial sepsis. *Shock* 2010; **34:** 281-90. *Pravdic D, Mio Y, Sedlic F, Pratt PF, Warltier*

DC, Bosnjak ZJ, Bienengraeber M. Isoflurane protects cardiomyocytes and mitochondria by immediate and cytosol-independent action at reperfusion. Br J Pharmacol 2010: 160: 220-32.

Reviews and Books

Fukuda N, Terui T, Ishiwata S, Kurihara S. Titin-based regulations of diastolic and systolic functions of mammalian cardiac muscle. J Mol Cell Cardiol 2010; 48: 876-81.

Uchino S. What is 'BEST' RRT practice? Contrib Nephrol 2010; 165: 244-50.

Uchino S. The meaning of transient azotemia. *Contrib Nephrol* 2010; **165:** 337–44.

Department of Rehabilitation Medicine

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General Summary

The main research topics of our department in the 2010 academic year focused on: 1) a new software program for computerized assessment; 2) basic research; 3) the Ability for Basic Movement Scale for Children (ABMS-C); and 4) repetitive transcranial magnetic stimulation (rTMS) plus occupational therapy (OT) for patients with upper limb hemiparesis after stroke and rTMS plus speech therapy for patients with aphasia after stroke.

Research Activities

A new software program for computerized assessment

The objective of this study was to prove the validity and reliability of 9 of the 29 tasks of the Higher Brain Functional Balancer (HBFB), a new software program for computerized assessment. Seventy apparently healthy elderly subjects (age, 63 to 86 years; 27 men and 43 women) participated in this prospective study. The association between the Mini-Mental State Examination (MMSE) and the HBFB was tested with Pearson's correlation coefficient analysis; internal consistency of the 9 tasks of the HBFB was checked with Cronbach's coefficient alpha (Cronbach's α), and the test-retest reliability of each task was established with intraclass correlation. For test-retest reliability, 9 tasks of the HBFB were administered to the subjects twice at a 1-month interval. The test-retest HBFB quotient, data on age, length of education, and results of the MMSE were recorded. Pearson's correlation coefficient analysis showed that the state of cognitive function according to the total scores of the MMSE correlated significantly with the total quotients of the HBFB (r=0.356, p=0.002). The 9 tasks of the HBFB had appropriate internal consistency (Cronbach's α =0.735). Test-retest reliability analysis indicated that the "Modified Trail Making Test," "Flashing- Light Memory," "Story," and "Route-99" tasks on the HBFB had fair-to-good reliability (intraclass correlation=0.364-0.742). However, reliability was poor with regard to the scores of the other 5 tasks. This study provides evidence for the validity of the total quotient of all tasks for the screening of total cognitive function and for the reliability of 4 of the 9 tasks from the HBFB with regard to cognitive function in elderly persons.

Basic research

Enterovirus 71 (EV71), a human enterovirus species A, is a major causative agent of hand, foot, and mouth disease and can also cause a wide spectrum of neurological dis-

We have recently identified human P-selectin glycoprotein ligand-1 (PSGL-1) as eases. a functional EV71 receptor and have demonstrated PSGL-1-dependent replication for some EV71 strains (PSGL-1-binding EV71 [EV71-PB]) in leukocytes. Four of 5 EV71-PB strains have replicated poorly in mouse L929 cells stably expressing human PSGL-1 (L-PSGL-1.1 cells); therefore, we compared the replication kinetics and the entire genomic sequence of 5 original EV71-PB strains and the corresponding EV71-PB variants (EV71-LPS), which were propagated once in L-PSGL-1.1 cells. In contrast to 4 of 5 original EV71-PB strains, all EV71-LPS variants efficiently replicated and induced cytopathic effects in L-PSGL-1.1 cells in a PSGL-1-dependent manner. Direct sequence analysis of the entire genome of the original EV71-PB strains and LPS variants identified several possible adaptive mutations during the course of replication in L-PSGL-1.1 cells, including a putative determinant of the adaptive phenotype in L-PSGL-1.1 cells at VP2-149, which was shared with 4 of the 5 LPS variants. The results suggest that an adaptive mutation(s), along with a PSGL-1-binding phenotype, facilitates the efficient PSGL-1dependent replication of EV71-PB strains in L-PSGL-1.1 cells

Predictive validity of the new ABMS-C scale

The objective of this pilot study was to test the validity and reliability of a new scale, the ABMS-C. A total of 45 pediatric patients with disabilities (age, 0.1 to 8.8 years; 29 boys and 16 girls) participated in this prospective study. To prove the validity and reliability of the ABMS-C, it was administered to subjects at a 2-week interval. In addition to the ABMS-C score, data on age, diagnosis, and results of the Gross Motor Function Classification System were recorded. Spearman's rank correlation coefficient analysis showed that the ability to perform basic movements according to the scores of each item and the total scores of the ABMS-C correlated significantly with the levels of the Gross Motor Function Classification System (r=-0.628-0.752; p<0.001). The 5 items on the ABMS-C had appropriate internal consistency (Cronbach's $\alpha=0.944$). Test-retest reliability analysis indicated that the "head control," "sitting," "locomotion on flat surface," "standing," and "walking" items of the ABMS-C had almost perfect reliability ($\kappa=0.865-1.000$). This study provides evidence for the validity and reliability of the ABMS-C for assessing the functional ability of disabled pediatric patients.

Low-frequency rTMS combined with intensive OT for upper limb hemiparesis

Six-day protocol: In-hospital combination treatment was provided for 5 patients who had had upper limb hemiparesis for more than 12 months after the onset of stroke. Over 6 consecutive days, each patient underwent 10 sessions of combination treatment with 1-Hz rTMS and intensive OT (one-on-one training and self-training). Motor function in the affected upper limb was evaluated with the Fugl-Meyer Assessment (FMA), Wolf Motor Function Test (WMFT), Ten-Second Test at admission, discharge, and 4 weeks after treatment. All patients completed the 6-day treatment protocol, and none showed any adverse effects throughout the treatment. At the end of treatment, improvements in the scores of the FMA, WMFT, and Ten-Second Test were found in all patients. No deterioration of the improved upper limb function was observed 4 weeks after the end of treatment. Our proposed protocol of combination treatment appears to be safe and feasible

for patients with poststroke upper limb hemiparesis, although the efficacy of the protocol needs to be confirmed in a large number of patients.

15-day protocol: Fifteen patients (age at study entry, 55±17 years; time after stroke, 57±55 months) with poststroke upper limb hemiparesis categorized as Brunnstrom stages 3 to 5 for hand-fingers were recruited. They were considered to have reached a plateau state at study entry, based on the lack of any increase in the FMA score in the previous 3 months. During the 15-day hospitalization, each patient underwent 22 sessions of 1-Hz rTMS to the contralesional cerebral hemisphere, followed by intensive OT (one-to-one training including shaping techniques and self-training). Upper limb motor function was evaluated with the FMA and the WMFT at admission and discharge. The spasticity of finger flexors, wrist flexors, and elbow flexors in the affected upper limb was also evaluated with the Modified Ashworth Scale. The 15-day protocol was well tolerated by all patients. At discharge, the FMA score was increased in all 15 patients (from 17 to 57 points to 18 to 61 points). Shortening of performance time on the WMFT was noted in 12 patients (from 44 to 1,584 seconds to 39 to 1,485 seconds). The Modified Ashworth Scale score for some flexor muscles decreased in 12 patients. In conclusion, our 15-day protocol of low-frequency rTMS combined with intensive OT appears feasible for improving motor function and for reducing spasticity in the affected upper limb in patients with poststroke hemiparesis.

Low-frequency rTMS combined with intensive speech therapy for aphasia

Two Japanese patients with poststroke sensory-dominant aphasia were studied. Both patients underwent 10 sessions of 20-minute low-frequency (1 Hz) rTMS to Wernicke's area during a 6-day hospitalization, followed by weekly outpatient rTMS treatment for 3 months. Language therapy was also provided through the period of inpatient and outpatient treatment. Language function was evaluated with the Token Test and the Standard Language Test of Aphasia at the start and end of inpatient treatment and the end of outpatient treatment. The therapeutic protocol was well tolerated throughout the inpatient and outpatient treatments, without any adverse effects. The scores of the Token Test and certain subcategories of the Standard Language Test of Aphasia increased in both patients after inpatient rTMS treatment. Persistent improvements of the scores were noted over the 3-month postdischarge period. The proposed protocol of long-term application of low-frequency rTMS to Wernicke's area and speech therapy is considered a safe and feasible therapeutic approach for patients with poststroke sensory-dominant aphasia.

Publications

Miyamura K, Nishimura Y, Abo M, Wakita T, Shimizu H. Adaptive mutations in the genomes of enterovirus 71 strains following infection of mouse cells expressing human P-selectin glycoprotein ligand-1. J Gen Virol 2011; 92: 287-91. Honda M (Nico Child Clin), Hashimoto K, Miyamura K, Goto H (LEDEX Corp), Abo M. Validity and reliability of a computerized cognitive assessment tool 'Higher Brain Functional Balancer' for healthy elderly people. Ninchi Shinkei Kagaku 2010; **12**: 191-7.

Kakuda Ŵ, Abo M, Uruma G, Kaito N, Watanabe M. Low-frequency rTMS with language therapy over a 3-month period for sensory-dominant aphasia: case series of two post-stroke Japanese patients. Brain Inj 2010; **24:** 1113-7.

Kakuda W, Abo M, Kobayashi K, Momosaki R, Yokoi A, Fukuda A, Ishikawa A, Ito H, Tomi**naga A.** Low-frequency repetitive transcranial magnetic stimulation and intensive occupational therapy for poststroke patients with upper limb hemiparesis: preliminary study of a 15-day protocol. *Int J Rehabil Res* 2010; **33**: 339-45.

Kakuda W, Abo M, Kobayashi K, Momosaki R, Yokoi A, Ito H, Umemori T. Low-frequency rTMS combined with intensive occupational therapy for upper limb hemiparesis after brain tumour resection. Brain Inj 2010; 24: 1505-10.

Ishikawa A, Kakuda W, Taguchi K, Uruma G, Abo M. The reliability and validity of a new subjective assessment scale for poststroke upper limb hemiparesis, the Jikei assessment scale for motor impairment in daily living (in Japanese). *Tokyo Jikeikai Ika Daigaku Zasshi* 2010; **125:** 159-67.

Yokoi A, Kakuda W, Fukuda A, Ito H, Tominaga A, Umemori T, Kameda Y, Ishikawa A, Abo M. Combination treatment of low-frequency rTMS and intensive occupational therapy for poststroke patients with upper limb hemiparesis: clinical protocol and results of neuto15 (in Japanese). Tokyo Jikeikai Ika Daigaku Zasshi 2011; 126: 79-89. Sawada H, Ishikawa A, Takekawa T, Kakuda W, Kawashima K, Abo M. Botulinum toxin type a injection combined with self-training proposed by occupational therapists for spastic upper limb hemiparesis after stroke (in Japanese). Tokyo Jikeikai Ika Daigaku Zasshi 2011; **126**: 99–109.

Kimura A¹, Abo M, Kawate N², Osako Y³, Suyama K³, Maeda T³, Uechi Y³, Iwasaki M³ ('Keio Univ, ²Showa Univ, ³Glaxo Smith Klein). Efficacy and safety of botulinum toxin type A in treating upper limb spasticity in poststroke patients: a multicenter, double-blind, placebo-controlled trial followd by an open-label trial (in Japanese). Jpn J Rehabil Med 2010; **47:** 714-27.

Kimura A¹, Abo M, Kawate N², Osako Y³, Suyama K³, Maeda T³, Uechi Y³, Iwasaki M³ ('Keio Univ, ²Showa Univ, ³Glaxo Smith Klein). Efficacy and safety of botulinum toxin type A in treating lower limb spasticity in poststroke patients: a multicenter, double-blind, placebo-controlled trial followed by an open-label trail (in Japanese). Jpn J Rehabil Med 2010; **47:** 626-36.

Department of Emergency Medicine

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General Summary

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

Research Activities

1. Supervision and development of ultrasound devices in the diagnosis and treatment of cerebrovascular disorders

- 2. Director of the Japan Neurotrauma Data Bank Committee
- 3. Prognostic value of heart fatty acid-binding protein for patients with chest symptoms in the emergency room
- 4. Research committee on higher cerebral function after traumatic brain injury
- 5. Research committee on impact biomechanics in automobile accidents (Society of Automotive Engineers of Japan, Inc.)

6. Published a revised edition of *Guidelines for the Treatment and Management of Severe Head Injury* (The Japan Society of Neurotraumatology)

- 7. Research group on traumatic intracranial hypotension
- 8. Management of Japan Advanced Trauma Evaluation and Care course

Publications

Murakami A¹, Takasugi H¹, Ohnuma S¹, Koide Y¹, Sakurai A², Takeda S, Hasegawa T³, Sasamori J³, Konno T³, Hayashi K³, Watanabe Y³, Mori K³, Sato Y¹, Takahashi A³, Mochizuki N², Takakura N⁴ (¹Tokyo Res Lab, ²Natl Cardiovasc Ctr Res Inst, ³Fukushima Res Lab, ⁴Osaka Univ). Sphingosine 1-phosphate (S1P) regulates vascular contraction via S1P3 receptor: investigation based on a new S1P3 receptor antagonist. Mol Pharmacol 2010; **77**: 704-13.

Otani K, Kasuga Y, Kimura Y, Mukaide M, Yanai H, Koyama T, Fujise K. Hepatits B surface antigen is a better monitor of infectivity compared with antibody to hepatitis B core antigen in hemodialysis patients. *Ther Apher Dial* 2010; **14**: 434-5.

Endo S, Okuno K, Kobayashi H, Ogawa T, Makuuchi H. Diagnostic multidetector-row computed tomography usefulness in intimal carotid artery cervical stab wound: a case report (in Japanese). *Rinsho Geka* 2010; **65**: 1159-62.

Kishimoto K, Hatano T, Egawa S, Miyake R, Ohasi K, Koyama T. Two cases of severe renal injury where silo closure was effective (in Japanese). Rinsho Hinyokika 2010; 64: 1017-20.

Reviews and Books

Ogagwa T. Current status of emergency medical service in Japan (in Japanese). *Shojinkai Igakushi* 2010; **49:** 1-8. Okuno K, Ohashi K, Ogawa T. Anticonvulsant drugs (in Japanese). Shokaki Geka 2010; **33**: 726-30.

Okuno K. Intracranial pressure management of severe traumatic brain injury (in Japanese). *Kyukyu Igaku* 2010; **34:** 1795-9.

Department of Endoscopy

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General Summary

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

Research Activities

Pharyngeal, esophageal, and gastric malignancies

1. Endoscopic diagnosis in esophagogastric neoplasia

Early detection and accurate diagnosis of premalignant and malignant lesions in the pharynx, esophagus, and stomach are essential for selecting the most appropriate therapeutic strategy for each patient. Our research utilizes 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 important 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 (×90) endoscope and an NBI light source, which provides detailed morphological information about the capillaries on the mucosal surface. We studied the clinical utility of NBI magnifying endoscopy for superficial neoplasms in the pharynx, esophagus, stomach, and duodenum. Our current studies focus on the development of algorithms for NBI technology which will allow histological type and tumor extent in gastric carcinoma to be predicted without biopsy. On the basis of our findings with magnified NBI, we have also developed a novel classification system for gastric cancer and demonstrated its advantages over conventional diagnostic systems in a prospective study. We joined a multicenter study using NBI magnifying endoscopy to detect superficial carcinoma in the pharynx and esophagus. Moreover, we performed a single-center study to compare NBI magnifying endoscopy with Lugol chromoendoscopy for detecting superficial carcinoma in the esophagus. We also aim to evaluate this technology for the early detection of precancerous changes in the specialized columnar epithelium that occurs in Barrrett's esophagus. Results of these studies have been reported at several conferences and have been published. Most recently, a magnifying endoscope and light source system equipped with NBI have been developed, and instruments now available for clinical use. We performed a comparative study of this novel NBI magnifying endoscopy and conventional high-definition magnifying endoscopy for detecting superficial carcinomas in the pharynx and esophagus.

2) AFI

Recently, an AFI endoscopic system has been developed to visualize autofluorescence emitted from the GI wall. In theory, AFI would allow the detection of premalignant lesions or early-stage malignant lesions that do not have a distinct endoscopic appearance on conventional white-light endoscopy. Although AFI still has a high false-positive rate, we found that AFI, in combination with conventional white-light imaging and NBI, can improve specificity.

3) Ultrathin endoscopy (transnasal endoscopy)

An ultrathin endoscope can reduce discomfort during endoscopic examination. However, the ultrathin endoscope has a lower image resolution than do conventional endoscopes, and, therefore has a higher risk of false-negative results. Accordingly, we found that rate of detection of gastric lesions was lower with ultrathin endoscopy than with high-resolution endoscopy. We are attempting to develop a method for 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 with endoscopic examination. These include lesions within the GI walls, such as submucosal tumors of the esophagus and stomach, and mediastinal and lymph-node lesions. In EUS-FNA, the real-time ultrasonographic images are used to precisely guide the biopsy needle into lesions. The tissues obtained with EUS-FNA are immediately examined for malignant cells by a cytologist or pathologist. We are now evaluating the safety and usefulness of this technique in ongoing studies.

2. Endoscopic treatment of esophageal and gastric malignancies

With recent advances in endoscopic diagnostic techniques and instrumentation, the indications for endoscopic therapy in early gastric and esophageal carcinomas have expanded. Research on the following endoscopic therapeutic modalities is now under way to standardize the use of these techniques for treating upper GI tract tumors.

1) New indications for endoscopic treatment and endoscopic submucosal dissection

Current indications for endoscopic mucosal resection (EMR) are limited by lesion size, depth, and histological type. Our recent efforts have focused on expanding the indications for endoscopic submucosal dissection (ESD) in the treatment of early gastric cancer, based on histopathological findings. We are also evaluating the potential new use of EMR for gastric cancers, including small, poorly differentiated adenocarcinomas lacking ulceration, well-differentiated adenocarcinomas 30 mm or smaller or confined to the mucosa, and carcinomas lacking submucosal microinvasion. The current indications for EMR include esophageal cancer, epithelial cancer (m1), and cancer partially invading the

lamina propria mucosae (m2) with a negligible risk of lymph-node metastasis. New indications for EMR now being evaluated include mucosal cancer invading the lamina muscularis mucosae (m3) and lesions with slight submucosal invasion within the inner third of the submucosal layer (sm1). At present, en bloc resection via ESD is considered necessary for the further development of endoscopic treatment. The 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 also evaluated the effectiveness of gastric acid-suppressing agents, which have been used empirically following endoscopic treatment, by monitoring intragastric pH after endoscopy. A blood culture study to evaluate the risk of sepsis and endotoxemia following ESD is now underway.

2) Treatments with innovative endoscopy systems. The multibending scope (M-scope) is a new type of endoscope that provides greater access to sites that are usually difficult to access. We have previously reported 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, regions that are not accessible with a conventional endoscope. Studies with an M-scope with magnifying capability are now underway to develop more accurate and safer procedures. Furthermore, clinical studies using a newly developed therapeutic endoscope (R-scope), with a special mechanism allowing the forceps to move laterally and vertically, in addition to the multibending function, are proceeding to advance the potential of endoscopic therapy. We have also performed several studies using natural orifice translumenal endoscopic surgery, including full-thickness resection, as current endoscopic treatments are directed only at mucosal diseases.

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

Many studies have demonstrated an association between *H. pylori* infection and the development of gastric cancer. However, there are still many unknown factors affecting this association. Because our department routinely performs endoscopic treatment for gastric cancer, clarification of these factors is important. Experiments 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 have also been exploring the roles of inducible nitric oxide synthase (iNOS) in the pathogenesis of *H. pylori*-associated diseases and have demonstrated that eradication of *H. pylori* plays important roles 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 using iodine staining, or Lugol chromoendoscopy, has enabled endoscopists to detect esophageal cancer at an early stage, thus improving patient prognosis. However, this technique is difficult to perform in such locations as the oropharynx and hypopharynx. The presence of metachronous or synchronous cancer in the oropharynx or hypopharynx has become the main factor adversely affecting the prognosis and quality of life of patients with esophageal cancer. Because detecting cancer at an early stage is important, we have found that the combination of magnifying endoscopy and the NBI system can be used to detect hard-to-find cancers at an early stage without the need for Lugol chromoendoscopy. A multicenter randomized, controlled study has examined the clinical value of this new combination endoscopy. In addition, we performed a study at our institution to evaluate the endoscopic characteristics of superficial carcinoma in the pharyngeal region. The results have reported at medical congresses and in English-language medical journals.

Functional disorders of the upper GI tract

The causes of gastroesophageal reflux diseases, including nonerosive reflux disease, and GI motility disorders are difficult to identify. To understand disease pathophysiology and choose effective treatments, it is important to establish methods to evaluate the hypersensitivity and dysmotility of the GI tract. Hence, we have developed a new method of evaluating esophageal function using a small-caliber endoscope. We have started basic experiments on esophageal motility and sensitivity to transform this technique from a research tool into a clinical tool.

Diagnosis and treatment of esophagogastric varices

We have recently used color-Doppler endoscopic ultrasonography (CD-EUS) to study the hemodynamics of the portal venous system in patients with esophagogastric varices. These studies have clarified several factors associated with an increased risk of recurrence of esophagogastric varices after endoscopic treatment. When all such factors are identified, we will be able to predict and prevent the early recurrence of varices after treatment. We have also started a study to confirm factors that exacerbate hemorrhagic gastritis and cardiac varices. CD-EUS is also highly accurate for detecting gastrorenal shunts, which can complicate the treatment of esophagogastric varices, and can visualize shunts in detail. Therefore, this diagnostic system would be extremely useful for selecting patients with esophagogastric varices who are candidates for interventional radiology and for predicting the efficacy of this treatment.

Enteroscopy and colonoscopy

1. Diagnostic techniques

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

Recently, the prevalence of colon cancer has markedly increased, particularly in Japan. In Europe and the United States, several reports of capsule endoscopy for examining the large intestine have been published. In Japan, we are collaborating with 6 hospitals to perform studies of capsule endoscopy to screen patients for neoplasms of the

colon.

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

2. Research in endoscopic interventions

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

3. Capsule endoscopy and enteroscopy

Capsule endoscopy is a minimally invasive endoscopic modality that can be used to detect lesions in areas of the small intestine unreachable with traditional push enteroscopy. Recently, particularly in Western countries, capsule endoscopy has been recommended as the first-line endoscopic examination for evaluating and managing obscure GI bleeding. We have performed capsule endoscopy for 109 patients since Japanese health insurance began covering the examination in April 2007. Our study, which was published in scientific journals, 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 for evaluating obscure GI bleeding by reevaluating 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), establishment of a standardized, systematic diagnostic algorithm for biliary and pancreatic diseases has become more important than ever. We are comparing the diagnostic accuracy of EUS-FNA, multidetector-row computed tomography, magnetic resonance cholangiopancreatography, and endoscopic retrograde cholangiopancreatography (ERCP) in hepatopancreatic diseases. Additionally we introduced second-generation contrast media for ultrasonic imaging in EUS diagnosis.

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

For the diagnosis of ampullary tumors of the duodenum, we have characterized mucosal surface structures in detail using NBI to magnify microstructures and to help determine whether a lesion is benign or malignant. Furthermore, convex array EUS examination 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 using endoscopic techniques in pancreatobiliary diseases

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

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

Palliative care

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

Publications

Yoshimura N, Goda K, Tajiri H, Ikegami M, Nakayoshi T, Kaise M. Endoscopic features of nonampullary duodenal tumors with narrow-band imaging. *Hepatogastroenterology* 2010; **57**: 462-7.

Imazu H, Uchiyama Y, Matsunaga K, Ikeda K, Kakutani H, Sasaki Y, Sumiyama K, Ang TL, Omar S, Tajiri H. Endoscopic features of nonampullary duodenal tumors with narrow-band imaging. Scand J Gastroenterol 2010; 45: 732-8.

Rey JF, Ogata H, Hosoe N, Ohtsuka K, Ogata N, Ikeda K, Aihara H, Pangtay I, Hibi T, Kudo S, Tajiri H. Feasibility of stomach exploration with a guided capsule endoscope. *Endoscopy* 2010; **42:** 541–5.

Sumiyama K, Tajiri H, Gostout CJ, Kawamura

M, Imazu H, Ohya TR, Ikeda K, Goda K, Saito S, Kato T. Chemically assisted submucosal injection facilitates endoscopic submucosal dissection of gastric neoplasms. *Endoscopy* 2010; **42:** 627-32.

Kato M, Kaise M, Yonezawa J, Toyoizumi H, Yoshimura N, Yoshida Y, Kawamura M, Tajiri H. Magnifying endoscopy with narrow-band imaging achieves superior accuracy in the differential diagnosis of superficial gastric lesions identified with white-light endoscopy: a prospective study. *Gastrointest Endosc* 2010; **72**: 523-9.

Kaneyama H, Kaise M, Arakawa H, Arai Y, Kanazawa K, Tajiri H. Gastroesophageal flap valve status distinguishes clinical phenotypes of large hiatal hernia. World J Gastroenterol 2010; 16: 6010-5. Tamai N, Matsuda K, Sumiyama K, Isshi K, Narimiya N, Tajiri H. Prophylactic hemostasis for postpolypectomy mucosal defect using endoclip under infrared imaging endoscopy. Surg Technol Int 2010; **19:** 91-6.

Ikeda K, Sumiyama K, Tajiri H, Yasuda K, Kitano S. Evaluation of a new multitasking platform for endoscopic full-thickness resection. *Gastrointest Endosc* 2011; **73:** 117-22.

Silva FB, Dinis-Ribeiro M, Vieth M, Rabenstein T, Goda K, Kiesslich R, Haringsma J, Edebo A, Toth E, Soares J, Areia M, Lundell L, Marschall HU. Endoscopic assessment and grading of Barrett's esophagus using magnification endoscopy and narrow-band imaging: accuracy and interobserver agreement of different classification systems (with videos). Gastrointest Endosc 2011; **73:** 7-14.

Sumiyama K, Utsunomiya K, Ohya T, Aihara H, Ikeda K, Imazu H, Tamai N, Nagano H, Ishinoda Y, Tajiri H. A pilot study on ultrasoundassisted liposuction of the greater omentum in porcine models. *Minim Invasive Ther Allied Technol* Epub 2011 Mar 14.

Reviews and Books

Sumiyama K, Tajiri H. Part 6: Diseases of the stomach 48. Gastric adenocarcinoma. In: Talley NJ, DeVault KR, Fleischer DE, editors. Practical gastroenterology and hepatology: Esophagus and stomach. West Sussex: Wiley-Blackwell; 2010. p. 374-83.

Department of Infection Control

Shoichi Onodera, Professor Masaki Yoshida, Assistant Professor Yasushi Nakazawa, Assistant Professor Seiji Hori, Professor Hiroshi Takeda, Assistant Professor

General Summary

Several clinical studies and 1 basic research study were performed in our department. Our retrospective clinical studies demonstrated several issues in patients with infectious diseases, catheter-related bloodstream infection, and antibiotic therapy. We will provide feedback to improve the outcome of cases of infectious diseases and to develop prospective clinical studies and basic research. On the other hand, we investigated biofilm formation using clinically isolated staphylococci. We will continue these clinical and basic research studies to obtain new evidence and to develop more effective treatments for infectious diseases.

Research Activities

Analysis of catheter-related bloodstream infections

Sixty-four cases (16%) of catheter-related bloodstream infections were identified among 384 cases of bloodstream infection at The Jikei University Hospital. Staphylococci, including methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci, were isolated from 31 of 50 patients with central venous catheters. On the other hand, Gram-negative bacilli, such as *Enterobacter* spp. and *Serratia* spp., were isolated from 8 patients with peripheral venous catheters. Effective strategies must be developed to prevent catheter-related bloodstream infections.

Clinical characteristics and risk factors for mortality in patients with bacteremia caused by Pseudomonas aeruginosa

In our hospital, we performed retrospective analyses to determine risk factors for mortality among patients with bacteremia caused by *Pseudomanas aeruginosa* (*P. aeruginosa*). A total of 134 patients with *P. aeruginosa* bacteremia were identified from April 2003 through March 2010. The 30-day mortality rate among all patients with *P. aeruginosa* bacteremia was 20.9%. The most common underlying disease was leukemia (20.9%), and the most common primary site of infection was the urinary tract (24.6%). Seventy-one patients (65.7%) were treated with an appropriate initial antimicrobial regimen for *P. aeruginosa* bacteremia. However, the 30-day mortality rate in these patients was similar to that in patients not given appropriate antibiotics. This study revealed that risk factors for 30-day mortality were thrombocytopenia and polymicrobial *P. aeruginosa* bacteremia (p<0.01). patients were men aged 22 to 42 years. The most common clinical features were fever and sore throat, followed by splenomegaly and skin eruption. The CD4-positive lymphocyte count was 100 to $635/\mu$ L, and the HIV RNA level was 1.9×10^5 to 9.6×10^6 copy/ mL. Acquired immunodeficiency syndrome developed in 2 patients; 1 patient had esophageal candidiasis, and another had pneumocystis pneumonia. Because clinical features were not specific and because antibodies against HIV were not detected in patients with acute HIV infection, we recommend that the polymerase chain reaction be used to diagnose acute HIV infection.

Biofilm formation of clinical isolated staphylococcus species

We analyzed the capacity of biofilm formation and the biofilm component *in vitro*, using the staphylococci isolated from patients at The Jikei University Hospital. Biofilm formation was observed in 29.2% (7 of 24 strains) of strains of methicillin-sensitive S. aureus (MSSA), 29.2% (7 of 24 strains) of strains of MRSA, and 25.0% (7 of 28 strains) of strains of Staphylococcus epidermedrs (S. epidermidis). Of the 7 biofilmforming staphylococci strains, 2 strains were induced by NaCl, and 5 strains were induced by glucose. Only 1 biofilm formed by MRSA was destroyed by a polysaccharide-degradative enzyme (dispersin B), but 4 biofilms formed by S. epidermidis were susceptible to dispersin B. On the other hand, a protein-degradative enzyme (proteinase K) destroyed 4 biofilms formed by MSSA and 4 formed by MRSA but only 2 biofilms of S. epidermidis. Seven of the 10 biofilms susceptible to proteinase K were destroyed by a DNAdegradative enzyme (DNase I). The frequency of biofilm formation did not differ markedly among the clinically isolated strains of MSSA, MRSA, and S. epidermidis. The biofilms of S. epidermidis were dependent on polysaccharides; on the other hand, the biofilms of S. aureus were dependent on proteins. These findings suggest that a large amount of extracellular DNA is contained in proteinaceous biofilms.

Comparison of susceptibility of P. aeruginosa to carbapenems

To compare the susceptibility of clinically isolated *P. aeruginosa* to 5 carbapenem antibiotics (imipenem, panipenem, biapenem, meropenem, and doripenem), minimum inhibitory concentrations (MICs) were determined with a broth microdilution method. A total of 566 clinical isolates of *P. aeruginosa* were collected at The Jikei University Hospital from January through December in 2009. The MICs of doripenem were lower than those of other carbapenems. In addition, doripenem might have antimicrobial activity against imipenem-resistant *P. aeruginosa*, because the MIC of doripenem needed to inhibit growth by 50% was 4 µg/mL among strains of imipenem-resistant *P. aeruginosa*.

Comparison of treatment with vancomycin, teicoplanin, or linezolid in patients with MRSA pneumonia in the intensive care unit

The aim of this retrospective study was to compare the results of treatment with vancomycin, teicoplanin, or linezolid in patients with MRSA pneumonia in intensive care units (ICUs) at The Jikei University Hospital. In ICUs, pneumonia due to MRSA was diagnosed in 29 patients. The pneumonia was treated with vancomycin in 22 patients, teicoplanin in 3 patients, and linezolid in 4 patients. Three of the 4 patients treated with linezolid showed rapid improvement. Our results suggest that linezolid should be the antibiotic of first choice for treating MRSA pneumonia in the ICU.

Publications

Hori S, Irimajiri S (Kasawaki Mun Hosp), Koido N (Kasawaki RA&IM Cli), Sunakawa K (Kitasato Inst Life Sci). Safety profile of pediatric tosufloxacin (in Japansese). Nihon Kagaku Ryoho Gakkai Zasshi 2010; **58 S-2**: 78-88.

Yoshikawa K, Okada H, Hasegawa T, Sakurai I, Kawaguchi Y, Onodera S. Clinical features and treatments of patients with urosepsis: comparison of community-aquired and hospital-aquired infections (in Japanese). Tokyo Jikeikai Ika Daigaku Zasshi 2010; **125**: 121-7.

Sato F, Iwase T, Tajima A, Shinji H, Mizunoe Y. Biofilm formation of clinical isolated Staphylococcus species (in Japanese). BACTERIAL ADHEREN & BIOFILM 2010; 23: 23-8.

Kizu J, Iwata S, Kusachi S, Sato J, Sato Y, Sandoh M, Takeda H, Tateda K, Hori S. Do doctors know of and use PK-PD-based antimicrobial agent dosage? (in Japanese) *Nihon Kagaku Ryoho Gakkai Zasshi* 2010; **58:** 460-5.

Kizu J, Hori S. Review of the literature on interaction of second-genration antihistamines with other drugs (in Japanese). *Iyakuhin Sogo Sayo Kenkyu* 2010; **34:** 23-31.

Kizu J, Maezawa K, Terashima T, Fukuda H, Akita H, Hori H. Examination of the prescription of antihistamines: based on a questionnaire survey involving practicing physicians (in Japanese). Arerugi Meneki 2010; 17: 2066-76.

Arakawa S, Kawai S, Hori S, Watanabe S, Totsuka K. A clinical phase III study of pazufloxacin in patients with sepsis (in Japanese). *Nihon* Kagaku Ryoho Gakkai Zasshi 2010; **58**: 650-63.

Kohno S, Aoki N, Kawai S, Niki Y, Watanabe A, Hori S, Watanabe S, Totsuka K. A clinical phase III study of pazufloxacin in patients with bacterial pneumonia. *Nihon Kagaku Ryoho Gakkai Zasshi* 2010; **58:** 664-80.

Reviews and Books

Hori S. Anti-fungal drugs, PK-PD (in Japanese). *Rinsho to Biseibutsu* 2011; **38:** 105-9.

Horino T. Prognostic factors and the role of interleukin-1 in bacteremia caused by Pseudomonas aeruginosa (in Japanese). *Nihon Kagaku Ryoho Gakkai Zasshi* 2010; **58**: 547-54.

Kato T. Non-AIDS defining malignancy (in Japanese). Nihon AIDS Gakkaishi 2010; **12:** 137-43.

Kato T. Fever with malignancy (in Japanese). Chiryo 2010; 92: 1954-7.

Hori S. Safety and drug interaction (in Japanese). In: Kono S, editor. How to use carbapenems. Osaka: Iyaku Janarusha; 2010. p. 54-65.

Department of Dentistry

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

General Summary

1. 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.

2. Basic studies of obstructive sleep apnea-hypopnea syndrome

To clarify the effects of obesity on the properties and volume of lingual muscles, we analyzed the accumulation of triacylglycerol and the diameter of myofibers in the lingual muscles of obese rats fed a high-fat diet.

Research Activities

Clinical studies of TMDs

1. Reliability of a questionnaire of pain-related limitations of daily function in Japanese patients with TMDs

Purpose: We have used a new questionnaire to examine pain-related limitations of daily function due to TMD (LDF-TMDQ) in Japanese patients. The LDF-TMDQ consists of 10 items and can estimate 3 latent variables: "Limitation of mouth opening," "Limitation in executing a certain task," and "Limitation of sleeping." We have already verified the construct validity and cross validity of the LDF-TMDQ. The purpose of this study was to assess the within-day reliability of the LDF-TMDQ in Japanese patients with TMD.

Methods: All outpatients were asked to complete the LDF-TMDQ before they were examined at their first visit. Patients who were found to have painful TMD were requested to complete the LDF-TMDQ again on the same day after they had returned home. A total of 103 consecutive patients with TMD were recruited, and 77 of these patients (75%) completed the LDF-TMDQ and were eligible for analysis. Test-retest reliability was assessed with intraclass correlation coefficients and Spearman's correlation coefficients using the software program IBM SPSS Statistics version 12 (IBM Corp., Armonk, NY, USA). Internal consistency was assessed with Cronbach's α .

Results: The intraclass correlation coefficients for test-retest reliability of "Limitation of mouth opening," "Limitation in executing a certain task," and "Limitation of sleeping" were 0.71, 0.71, and 0.83, respectively. Spearman's correlation coefficients were 0.69, 0.71, and 0.79 (p<0.001), respectively. Cronbach's α values were 0.83, 0.83, and 0.91, respectively.

Conclusion: This study shows that LDF-TMDQ has excellent within-day reliability.

2. Background of patients with false-positive and false-negative results on screening

test for TMDs

Purpose: To investigate the background of patients with false-positive (FP) and false-negative (FN) results on 1-item screening tests (binary scale) for TMDs.

Methods and subjects: We analyzed 1,225 dental patients (666 males and 559 females). Patients with FP results were those in whom TMD was diagnosed with a 1-item screening test but not with clinical evaluation, and patients with FN results were those in whom TMD was not diagnosed with a 1-item screening test but was diagnosed with clinical evaluation. We investigated the differences of these definite diagnosis constitutions between patients with FP results and those with true-negative (TN) results. According to a 4-item questionnaire for TMD (disturbance of mouth opening, pain on jaw opening or closing or both, deviation opening, and pain on chewing hard food) on a 5-point numeric rating scale, we compared the scores of 4 items between the FP and TN groups, the FN and true-positive (TP) groups, and the FP and FN groups.

Results: The percentages of subjects with inflammation and dental caries were similar in the FP group and the TN group. The FP group had significantly higher total scores for the 4 items than did the TN group. The FN group had significantly lower total scores for jaw opening pain or closing pain or both than did the TP group.

Conclusion: The rates of inflammation and dental caries in the FP group were as high as in the TN group. The FN group had weaker pain than the PT group.

3. Investigation of contributing factors associated with TMDs in the working population Purpose: The purpose of this study was to extract contributing factors related to TMDs in the working population through a questionnaire survey.

Methods: We administered a questionnaire for TMD screening to determine contributing factors to 2,723 employees of company A and obtained valid responses from 2,203.

Results: Of the 2,203 employees, 362 were considered to have TMD (16.4%). This rate was higher than in the general population in Japan. The total scores for both psychosocial factors and habitual behaviors were significantly higher in persons with TMD than in persons without TMD. Multivariate logistic regression analysis showed the following to be significant factors contributing to TMD: increases in anxiety, fatigue, and tooth-contacting habit and symptoms upon waking in men, and an increase in fatigue and symptoms upon waking in women.

4. A randomized clinical trial of treatment for temporomandibular joint disc displacement

Of the various conservative treatments available for TMDs, we believe that therapeutic exercise leads to a good prognosis, especially for anterior disc displacement without reduction. Because its effectiveness has not been extensively evaluated, we performed a comparative study to verify the hypothesis that treatment efficacy does not differ between exercise and occlusal splints. Fifty-two patients with anterior disc displacement without reduction were randomly assigned to a splint treatment group or a joint mobilization self-exercise treatment group. Four outcome variables were evaluated: 1) maximum mouth opening range without pain and 2) with pain, 3) current maximum daily pain intensity, and 4) limitation of daily functions. All outcome variables showed significant improvements after 8 weeks of treatment in both groups. However, the mouth opening range increased more in the exercise group than in the splint group. This result demonstrates

that therapeutic exercise leads to earlier recovery of jaw function than does splint application.

5. Interview of volunteer patients for collecting patient questions in the clinical guideline for TMDs

We performed personal interviews of volunteer patients who previously had symptoms of TMDs to collect Patient Questions for a course for establishing guidelines for the initial treatment of TMD. We recruited subjects (known hereafter as "informants") by placing advertisements in 2 newspapers. After careful consideration, the study committee chose 10 persons from among 19 applicants as informants. The interviews were performed in a semistructured manner in an private room especially equipped for interviews. The answers from informants were tape-recorded and converted into oral sentences. From these sentences, important words and phrases were extracted and compiled by means of text mining. In regard to recognition of TMD, 4 informants answered "a disease in which the jaw is displaced," and 4 informants answered "a disease that occurs if one's occlusion is poor." These were the most answers in all, respectively. However, only 1 informant had received an explanation about TMD from her dentist. Although 3 informants of the 10 had undergone mouthpiece therapy, they had not received explanations about the necessity of or the reason for this therapy. Six informants were satisfied with their treatments, whereas 2 informants were dissatisfied. The treatments for joint noise, mouth-opening restriction, and pain most often chosen by informants were, in descending order, mouth-opening exercises, massage, compress, jaw rest, chiropractic, and mouthpiece. This investigation showed us that all informants had only superficial knowledge of pathologic conditions and treatment methods. Our findings suggest that dentists do not provide enough information about pathologic conditions and treatments for TMD.

6. Relations of sleep and headache in patients with TMDs

One-quarter to one-third of Japanese have headaches with unusual symptoms, and most have tension headaches. In 2005, we found that the prevalence of headache symptoms in 542 patients with painful TMDs was 49.1% and reported about the relation between headache and job type at the annual meeting of the Japanese Society for Temporomandibular Joint, but the relation of headache with sleep has not been investigated.

Purpose: We examined the relation between headache and sleep disturbance in patients with TMD.

Methods: After we obtained informed consent from 100 patients with painful TMD at their first examination, we asked them to complete evaluation questionnaire every 4 weeks. We continued to collect questionnaires from patients until they stopped their visits.

Result: The number of patient registered at the first examination was 100, and the percentage of patients with headache symptoms at the first examination was 43%. The male: female sex ratio was 43 : 57, and the average age was 37 ± 14.2 years. We analyze an anxiety classification and a dejection classification of number of going to hospital, sleep disturbance, and the Hospital Anxiety and Depression Scale with Clause 2 logistic regression analysis to analyze the presence or absence of headache, the dejection degree (odds ratio, 2.083) in women and sleep disturbance (odds ratio 2.551) was of choice in men with sleep disturbance (odds ratio: 1.564). Patients with headache symptoms were more likely to have sleep disturbance than were patients without headache symptoms (Mann-Whitney U test) with the presence or absence of headache because sleep disturbance was chosen by men and women.

Conclusion: In patients with painful TMD, we found a relation between headache and sleep disturbance.

7. Changes in headache in patients with TMD

Purpose: To clarify how headache changes during treatment for TMD.

Methods: After we obtained informed consent from 100 patients with painful TMD at their first examination, we asked them to complete evaluation questionnaire every 4 weeks. We continued to collect questionnaires from patients until they stopped their visits.

Result: The number of patients registered at the first examination was 100, and the percentage of patients with headache symptoms at the first examination was 43%. The male: female sex ratio was 43:57, and the average age was 37 ± 14.2 years. Women were observed more, as the relation between the presence of the headache and the sex differences of the headache. The prevalence of headache tended to decrease for 4 weeks after the first examination, but there were few subsequent changes. Headaches described as "extremely strong" were often absent during these 4 weeks, and the prevalence of "strong to moderate" headaches gradually decreased. As a result that we analyze an anxiety classification and a dejection classification of contents of operation work, sleep and Hospital Anxiety and Depression Scale using by Clause 2 logistic regression analysis to analyze for the presence or absence of headache, difficulty in falling asleep, input work, dejection degree were won characteristics, and anxiety degree was of choice in men.

Conclusion: Headaches may have been relieved by treatment for TMD by attention of the everyday life including operation work.

Basic studies of obstructive sleep apnea-hypopnea syndrome

1. Changes in the lingual muscles of rats with obesity induced by a high-fat diet

Purpose: To clarify the effects of obesity on the properties and volume of lingual (genioglossus and geniohyoid) muscles in rats.

Methods: We analyzed the accumulation of triacylglycerol and the diameter of myofibers in the lingual muscles using histochemical studies and examined myosin heavy chain composition using real-time polymerase chain reaction in rats fed a high-fat diet for 10 weeks.

Results: In the genioglossus and geniohyoid muscles, the percentage of oil droplet areas in obese rats were 3.6 and 2.5 times greater than those in control rats, respectively (p<0.025). The diameters of slow myofibers in the genioglossus and geniohyoid muscles were approximately 20% greater in the obese rats than in control rats (p<0.0001), whereas the diameters of fast myofibers in the geniohyoid muscle were approximately 10% greater in obese rats than in control rats (p<0.0001). No significant difference was found between the obese rats and control rats in the expression of any of the myosin heavy chain isoforms studied in any of the muscles examined.

Conclusion: A high-fat diet induces fat deposition in myofibers and affects the structure

of the lingual (genioglossus and geniohyoid) muscles.

Publications

Haketa T^I, Kino K^I, Sugisaki M, Takaoka M^I, Ohta T^I (^ITokyo Med Dent Univ). Randomized clinical trial of treatment for TMJ disc displacement. J Dent Res 2010: **89:** 1259-63.

Saito T, Yamane A¹, Kaneko S¹, Ogawa T¹, Ikawa T¹, Saito K¹, Sugisaki M ('Tsurumi Univ). Changes in the lingual muscles of obese rats induced by high-fat diet feeding. Arch Oral Biol 2010; 55: 803-8.

Kuruma E, Sugisaki M, Kino K (Tokyo Med Dent Univ), Tamai K, Saito T, Hayashi K. Reliability of questionnaire on the pain-related limitations of daily function in Japanese patients with temporomandibular disorders (in Japanese). Nihon Gakukansetsu Gakkai Zasshi 2010; **22:** 176-80.

Kino K¹, Kakudo K², Sugisaki M, Hoshi K³, Yuasa H⁴, Matsuka Y⁵, Saito T, Nishiyama A¹ (¹Tokyo Med Dent Univ, ²Osaka Dental Univ, ³Kitasato Univ, ⁴Tokai Municipal Hosp, ⁵Okayama Univ). Interview of volunteer patients for collecting patient question in the clinical guideline for temporomandibulara disorders (in Japanese). Nihon Gakukansetsu Gakkai Zasshi 2010; 22: 151-7.

Department of Transfusion Medicine

Yasutaka Hoshi, Professor Yoko Kato, Assistant Professor Tetsunori Tasaki, Associate Professor

General Summary

1. The quality and safety of blood products supplied by blood centers in Japan are extremely high, and the medical staff of every hospital is required to use these products properly. If an adverse event occurs in a patient who has received a transfusion, prompt treatment should be followed by an investigation of the cause to prevent a similar event from happening. To implement appropriate and immediate actions after transfusion-related adverse events, a preliminary online hemovigilance system was developed in 2007 that involved 7 university hospitals, including The Jikei University Hospital, and 5 smaller hospitals. Data were collected at the National Institute of Infectious Diseases and analyzed every 2 months. This system is expected to contribute to transfusion safety, not only for the 12 participating hospitals, but also for other hospitals that use blood components in Japan.

2. In pediatric transfusion medicine, the use of syringes as blood containers and transfusion of irradiated red blood cells are problems requiring investigation. We are concerned about the quality and functional changes of blood components stored in syringes and about the high potassium levels seen in irradiated units of red blood cells.

3. In Japan, red cell concentrates manufactured in blood centers using the additive solution mannitol-adenine-phosphate are required to be used within 3 weeks, although until 1995, the shelf life was 6 weeks. The main reason for the change was concern over contaminating bacteria, such as *Yersinia enterocolitica*. We are interested in increasing the storage time and have started a study to determine the risk of bacterial contamination and the cost-effectiveness of longer storage times.

4. Predeposit autologous transfusion is considered the ideal method of blood transfusion. Recombinant erythropoietin (r-EPO) is used to accelerate erythropoiesis for donorpatients with anemia and enables the preoperative collection of sufficient amounts of autologous blood. Use of r-EPO for autologous blood donation in the elderly is controversial because of concerns over adverse effects, such as hypertension, and cost-effectiveness. Therefore, definite criteria for the use of r-EPO in elderly donors should be established.

Research Activities

1. According to data from the online reporting system from 2007 through 2010, the overall incidence of adverse events per transfusion bag was 1.47%. The incidence of adverse events was significantly higher for platelet concentrates (4.16%) than for red blood cells (0.66%) or fresh-frozen plasma (0.93%). This study was reported by Odaka of the National Institute of Infectious Diseases.

2. Oxygen concentrations decreased rapidly (within 1 hour after preparation) in platelet concentrates stored in a syringe from which the air had been removed. Kataoka and Takahashi at Nara Medical University reported that with increasing pCO₂ and lactate levels, platelet aggregation induced by ADP was reduced.

Because potassium levels in irradiated red cell units increase rapidly, caution should be used when this blood is transfused into children. Especially for very low birth weight infants with immature renal function, fresher blood should be irradiated just before being released for transfusion. Otherwise the use of a transfusion filter with a potassium adsorption column is recommended as an alternative to prevent a sudden increase in the potassium level. Tasaki and Hoshi reported the strategy of transfusion for neonates with the support of a grant from the Ministry of Health, Labour and Welfare of Japan.

3. Because bacterial contamination in blood components is a serious problem, the incidence is being carefully investigated for various types of blood products to justify the extension of the storage period from 3 weeks to 6 weeks. This work is being supported by Grants-in Aid for Scientific Research (C).

4. Obvious adverse events have not been observed with the use of r-EPO in elderly patients. However, r-EPO is extremely expensive, and caution should be taken regarding cost-effectiveness and adverse effects of r-EPO when used for autologous blood donation.

5. Guidelines for the safe management of granulocyte transfusion have been reported by Dr. Osaka of Juntendo University Hospital. He is a member of the Granulocyte Transfusion Study Group.

Reviews and Books

Ohsaka A¹, Kikuta A², Ohto H², Ohara A³, Ishida A⁴, Osada K, Tasaki T, Kamitamari A⁵, Iwai A⁶, Kai S⁷, Maekawa T⁸, Hoshi Y (¹Juntendo Univ, ²Fukushima Med Univ, ³Toho Univ, ⁴Tachikawa Hosp, ⁵Sasebo City Hosp, ⁶Iwakuni Children's Hosp, ⁷Hyogo Coll Med, ⁸Kyoto Univ); Japan Society of Transfusion Medicine and Cell Therapy, Granulocyte Transfusion Task Force. Guidelines for safety management of granulocyte transfusion in Japan. Int J Hematol 2010; **91**: 201-8.

Institute of DNA Medicine Department of Gene Therapy

Toya Ohashi, Professor and Director

Hiroshi Kobayashi, Assistant Professor

General Summary

Our purpose is to develop treatments for intractable diseases, including genetic diseases, cancer, and diabetes. We performed various studies and investigations this year. Below, we 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 enzyme of Krabbe disease and mucopolysaccharidosis (MPS) VII and administered these vectors to newborn model mice. For Krabbe disease, we detected increased body weight and delayed onset of clinical symptoms but no effect on severe progressive symptoms. For MPS VII, effects on body weight and life span were observed, and copies of lentiviral vector DNA were detected in several organs, including the brain, of the treated neonatal model mice, a finding that indicates efficient long-term expression. We have started to make a recombinant lentivirus for a new gene therapy for MPS II.

2. Pathophysiological analysis of Pompe disease

We analyzed the signaling pathway of endoplasmic reticulum stress-induced autophagy in fibroblasts from patients with Pompe disease. We found that treatment with SB203580, an inhibitor of p38 mitogen-activated protein kinase, significantly inhibits induction of autophagy, whereas no effect is observed following treatment with other mitogen-activated protein kinase inhibitors. Furthermore, we demonstrated phosphorylation of p38 by endoplasmic reticulum stress in patient fibroblasts.

3. Immune tolerance induction for enzyme replacement therapy

This year we worked to develop immune tolerance induction for enzyme replacement therapy for Pompe disease. Parenteral administration of an anti-CD3 antibody induced immune tolerance against infused enzyme in various mouse strains, including Pompe disease model mice. In addition, the anti-CD3 antibody prevented a lethal hypersensitivity reaction to the enzyme infusion. The effect described above persisted for up to 20 weeks, even if the enzyme was repeatedly infused. The anti-CD3 antibody prevented increases of enzyme-specific immunoglobulin (Ig) E antibodies and IgG antibodies. The anti-CD3 antibody is also effective for preexisting antibodies against the enzyme. The CD4⁺ and CD8⁺ effector cells and CD4⁺ CD25⁺ regulatory T cells decreased with the anti-CD3 antibody. The immune-tolerant effects of the anti-CD3 antibody. This observation indicates that CD4⁺ CD25⁺ cells play a key role in the immunosuppressive effects of the

anti-CD3 antibody.

4. Antitumor effect and application to gene therapy of nafamostat mesilate for fatal gastrointestinal cancers

Recent studies have demonstrated that nuclear factor (NF)- κ B plays important roles in the regulation of cell apoptosis, inflammation, and oncogenesis. Inhibition of NF- κ B is a potential new strategy for the treatment of cancers. We have previously reported that nafamostat mesilate, a serine-protease inhibitor that is widely used to treat pancreatitis and disseminated intravascular coagulation and is used as an anticoagulant for hemodialysis in Japan, inhibits NF- κ B activation and induces apoptosis of pancreatic cancer. Moreover, we showed that addition of nafamostat mesilate promotes gemcitabine-or paclitaxel-induced apoptosis through the inhibition of NF- κ B activation of pancreatic cancer. The clinical usefulness of gemcitabine combined with nafamostat mesilate for patients with unresectable pancreatic cancer has been examined in a phase II study. Recently, we evaluated the antitumor effects of nafamostat mesilate on other gastrointestinal cancers.

Gene therapy combining gemcitabine with an adenoviral vector expressing tumor necrosis factor-alpha (TNF- α) is a new therapeutic approach for pancreatic cancer. However, such a combination therapy is limited owing to activation of NF- κ B by TNF- α and gemcitabine. We hypothesize that the addition of nafamostat mesilate will enhance the antitumor effect of combination therapy with TNF- α gene delivery and gemcitabine for pancreatic cancer.

5. Gynecologic Oncology

1) Cyclin D1 is a prognostic indicator in advanced serous ovarian cancer

We have previously reported that high-resolution oligonucleotide copy number analysis shows that cyclin E1 gene (CCNE1) amplification is strongly associated with treatment resistance in serous epithelial ovarian cancer (EOC). We focused on 66 advanced cases of serous EOC and investigated the associations between the expression of G1-S-phase regulatory proteins and clinicopathological variables. Immunohistochemical analysis for cyclin D1, pRb, p16,p53, p27^{Kip1}, p21^{Waf1/Cip1}, and cyclin E was performed with formalin-fixed tissue sections of primary surgical specimens. Univariate analysis showed that overexpression of cyclin D1 was correlated with reduced progression-free survival (p=0.00062) and overall survival (p=0.00037). Multivariate analysis identified overexpression of cyclin D1 (p=0.0019), reduced expression of p27^{Kip1} (p=0.042), and residual tumor volume (p=0.0092) as independent predictors of overall survival. Overexpression of cyclin D1 (p=0.011) and residual tumor volume (p=0.006) were significantly associated with first-line chemosensitivity. In advanced serous EOC, overexpression of cyclin D1 contributed largely to a poor prognosis due, perhaps in part, to chemoresistance. Cyclin D1 is a possible target for overcoming the refractory nature of advanced serous EOC.

2) Copy number analysis identifies novel interactions between genomic loci in ovarian cancer

We obtained genome-wide copy number alteration data from 4 different single nucleotide polymorphism array platforms, with a final data set of 398 ovarian tumors, mostly of the serous histological subtype. The large data set enabled refinement of minimal regions

and identification of rare amplicons, such as those at 1p34 and 20q11. We performed a novel co-occurrence analysis to assess cooperation and exclusivity of copy number alterations and analyzed their relationship to patient outcome.

Positive associations were identified between gains on 19q and 20q, gain of 20q and loss of X, and between several regions of loss, particularly 17q. We found weak correlations of genomic loci, such as 19q12, with clinical outcome.

3) IL-6-STAT3-HIF signaling and therapeutic response to the angiogenesis inhibitor sunitinib in ovarian clear cell cancer

We analyzed the most comprehensive gene expression and copy number data sets to date to identify potential therapeutic targets of ovarian clear cell adenocarcinoma (OCCA). Analyses of gene expression and DNA copy number were performed with primary human OCCA tumor specimens, and the findings were confirmed with immunohistochemical studies of tissue microarrays. We found specific overexpression of the IL-6-STAT3-HIF (interleukin 6 signal transducer and activator of transcription 3 hypoxiainduced factor) pathway in OCCA tumors compared with that in high-grade serous cancers. Expression of parathyroid hormone-like hormone and high levels of circulating interleukin 6 in patients with OCCA may explain the frequent occurrence of hypercalcemia of malignancy and thromboembolic events in OCCA. We described amplification of several receptor tyrosine kinases, most notably MET, which are potential therapeutic targets. We reported sustained clinical and functional imaging responses in 2 patients with chemotherapy-resistant OCCA who were treated with sunitinib and found significant parallels with renal clear cell cancer. Our findings highlight important therapeutic targets in OCCA, suggest that more extensive clinical trials with sunitinib in OCCA are warranted, and provide significant impetus to the growing realization that OCCA is molecularly and clinically distinct from other forms of ovarian cancer.

6. Islet biology and molecular medicine in diabetes mellitus

Disassembly and degradation of islet cells are major obstacles in the analysis of isolated islets *in vitro*, possibly due to ablation of the innervating vessels and nerves. To address this problem, we have developed a novel method for long-term maintenance of basic structures and functional analysis of islets of Langerhans using Matrigel basement membrane matrix (BD Biosciences, San Jose, CA, USA) and subcutaneous transplantation. Islets isolated from mice were mixed with Matrigel and was subjected to subcutaneous allograft. Grafts excised 24 hours after transplantation showed almost normal morphology as islets. After 10 days, the transplanted islets showed lobulated structures, but neovessels were observed within the islets. These results suggest that the subcutaneous transplantation method with Matrigel to maintain isolated islets could lead to highly functional analysis of isolated islets.

Publications

Fujiwara Y, Shiba H, Furukawa K, lida T, Haruki K, Gocho T, Wakayama S, Hirohara S, Ishida Y, Misawa T, Ohashi T, Yanaga K. Glasgow prognostic score is related to blood transfusion requirements and post-operative complications in hepatic resection for hepatocellular carcinoma. Anticancer Res 2010; **30**: 5129-36. Furukawa K, lida T, Shiba H, Fujiwara Y, Uwagawa T, Shimada Y, Misawa T, Ohashi T, Yanaga K. Anti-tumor effect by inhibition of NF- kappaB activation using nafamostat mesilate for pancreatic cancer in a mouse model. *Oncol Rep* 2010; **24:** 843-50.

Kobayashi H, Shimada Y, Ikegami M, Kawai T, Sakurai K, Urashima T, Ijima M, Fujiwara M, Kaneshiro E, Ohashi T, Eto Y, Ishigaki K, Osawa M, Kyosen SO, Ida H. Prognostic factors for the late onset Pompe disease with enzyme replacement therapy: from our experience of 4 cases including an autopsy case. *Mol Genet Metab* 2010; **100**: 14–9.

Kyosen SO, lizuka S, Kobayashi H, Kimura T, Fukuda T, Shen J, Shimada Y, Ida H, Eto Y, Ohashi T. Neonatal gene transfer using lentiviral vector for murine Pompe disease: long-term expression and glycogen reduction. *Gene Ther* 2010; **17:** 521-30.

Gheisari Y, Yokoo T, Matsumoto K, Fukui A, Sugimoto N, Ohashi T, Kawamura T, Hosoya T, Kobayashi E. A thermoreversible polymer mediates controlled release of glial cell line-derived neurotrophic factor to enhance kidney regeneration. *Artif Organs* 2010; **34**: 642-7.

Kobayashi H, Takahashi-Fujigasaki J, Fukuda T, Sakurai K, Shimada Y, Nomura K, Ariga M, Ohashi T, Eto Y, Otomo T, Sakai N, Ida H. Pathology of the first autopsy case diagnosed as mucolipidosis type III α/β suggesting autophagic dysfunction. *Mol Genet Metab* 2011; **102**: 170-5. Fujiwara Y, Shiba H, Furukawa K, Iida T, Sakamoto T, Gocho T, Wakiyama S, Hirohara S, Ishida Y, Misawa T, Ohashi T, Yanaga K. Perioperative change in white blood cell count predicts outcome of hepatic resection for hepatocellular carcinoma. J Hepatol 2010; **17**: 892-7.

lida T, Shiba H, Misawa T, Ohashi T, Eto Y, Yanaga K. Immunogene therapy against colon cancer metastasis using an adenovirus vector expressing CD 40 ligand. *Surgery* 2010; **148**: 925-35.

Tajima A, Ohashi T, Hamano S, Higurashi N, Ida H. Gaucher disease patient with myoclonus epilepsy and a novel mutation. *Pediatr Neurol* 2010; **42:** 65-8.

Togawa T, Kodama T, Suzuki T, Sugawara K, Tsukimura T, Ohashi T, Ishige N, Suzuki K, Kitagawa T, Sakuraba H. Plasma globotriaosylsphingosine as a biomarker of Fabry disease. *Mol Genet Metab* 2010; **100:** 257-61.

Meng XL, Shen JS, Kawagoe S, Ohashi T, Brady RO, Eto Y. Induced pluripotent stem cells derived from mouse models of lysosomal storage disorders. *Proc Natl Acad Sci U S A* 2010; **107:** 7886-91.

Sasaki T, Hiki Y, Nagumo S, Ikeda R, Kimura H, Yamashiro K, Gojo A, Saito T, Tomita Y, Utsunomiya K. Acute onset of rheumatoid arthritis associated with administration of a dipeptidyl peptidase-4 (DPP-4) inhibitor to patients with diabetes mellitus. Diabetol Int 2010; 1: 90-2.

Institute of DNA Medicine Department of Oncology

Mikio Zeniya, Professor Junko Yamada, Associate Professor Sadamu Homma, Associate Professor Shigeo Koido, Associate Professor

General Summary

Basic and clinical studies of cancer immunotherapy have been performed in our laboratory.

Research Activities

Phase I clinical study of immunotherapy against advanced pancreatic and biliary tract cancers by vaccination with dendritic cells pulsed with Wilm's tumor 1 peptide combined with gemcitabine

In this clinical study, dendritic cells (DCs) will be generated in the cell-processing center of the Shimbashi campus from peripheral blood mononuclear cells (PBMCs) obtained from patients of Kashiwa Hospital. The DCs will then be returned to Kashiwa Hospital and used as a therapeutic agent. We performed a dry run trial for the cell transfer 3 times using PBMCs and DCs obtained from a healthy volunteer and found that the cells could be transferred under the strictly controlled conditions to prevent microbial contamination and cell degradation.

Generation of cancer vaccine targeting tumor vessels from induced pluripotent stem cells Induced pluripotent stem (iPS) cells differentiated to progenitors of vascular cells showed a gene expression profile similar to that of tumor endothelial cells. Vaccination of mice with DCs pulsed with the lysate of vascular progenitor cells demonstrated potent antitumor activity against the challenge of CMS-4 tumor cells and lengthened survival. Small tumors formed subcutaneously in the immunized mice elicited significant suppression of tumor vessel development. Cancer vaccines targeting tumor vessels could be generated from iPS cells.

Immunotherapy with a DC and glioblastoma fusion cell vaccine

A clinical study of immunotherapy using a fusion cell vaccine of autologous DCs and malignant glioblastoma cells combined with temozoramide treatment has been performed for several years. Its safety and significant preventative effects against the postoperative recurrence of glioblastomas were demonstrated.

Exploitation of a novel cancer vaccine based on evolutionary molecular engineering Several artificial proteins composed of antigenic peptides of MHC class I and II and protein-stabilizing alpha-helix structure from ovalbumin were synthesized with an evolutionary molecular engineering technique. Because an immune response to the synthesized protein could be successfully induced in mice without adjuvant, this protein engineering technique might be used to generate new cancer vaccines that could elicit immune responses to human cancer antigens.

Generation of cancer vaccine from novel tumor markers for prostate cancer

Several proteins identified as novel candidate markers for prostate cancer by proteomics analysis have been evaluated for clinical application. Antigenic peptides on MHC class I molecules from human prostate cancer cells were examined for generation of peptide vaccines against prostate cancer by mass spectrometric analysis.

Publications

Ito M, Suzuki H, Sagawa Y, Homma S. The identification of a novel Paneth cell-associated antigen in a familial adenomatous polyposis mouse model. *Biochem Biophys Res Commun* 2010; **400:** 548-53.

Nagasaki E, Takahara A, Koido S, Sagawa Y, Aiba K, Tajiri H, Yagita H (Juntendo Univ), Homma S. Combined treatment with dendritic cells and 5-fluorouracil elicits augmented NK cellmediated antitumor activity through the tumor necrosis factor-alpha pathway. J Immunother 2010; **33:** 467-74.

Koido S, Hara E, Homma S, Namiki Y, Komita H, Takahara A, Nagasaki E, Ito M, Sagawa Y, Mitsunaga M, Uchiyama K, Satoh K, Arihiro S, Ohkusa T, Gong J, Tajiri H. Dendritic/pancreatic carcinoma fusions for clinical use: comparative functional analysis of healthy-versus patientderived fusions. *Clin Immunol* 2010; **135**: 384-400. Saeki C, Nakano M, Takahashi H, Saito S, Homma S, Tajiri H, Zeniya M. Accumulation of functional regulatory T cells in a actively inflamed liver in mouse dendritic cell-based autoimmune hepatic inflammation. *Clin Immunol* 2010; **135**: 156-66.

Reviews and Books

Koido S, Homma S, Hara E, Namiki Y, Okusa T, Gong J, Tajiri H. Antigen-specific polyclonal cytotoxic T lymphocytes induced by fusions of dendritic cells and tumor cells. J Biomed Biotech 2010; **2010**: 752381.

Koido S, Homma S, Hara E, Namiki Y, Takahara A, Komita H, Nagasaki E, Ito M, Ohkusa T, Gong J, Tajiri H. Regulation of tumor immunity by tumor/dendritic cell fusions. *Clin Dev Immunol* 2010; **2010**: 516768.

Institute of DNA Medicine Department of Molecular Genetics

Hisashi Yamada, Professor and Director

Takeshi Kawano, Assistant Professor

General Summary

Most biological phenomena are explained as a sequence of molecular genetic events. Molecular genetics is also revealing the causes of many diseases. Based on this knowledge, clinical medicine is also markedly changing. The diseases we are focusing on are malignant tumors, including hematological and pediatric malignancies. We are also investigating spinal muscular atrophy, Alzheimer's disease, and retinal diseases. Molecular pharmacological studies of anticancer agents are another part of our research. We are investigating these subjects from the viewpoint of molecular genetics to develop new diagnostic processes and treatments.

Research Activities

Exploring hematopoietic and pediatric malignancies

Most clinical events of cancers are explained on the basis of cancer stem cell theory, which assumes cancer stem cells are at the top of a cancer hierarchy. According to the classical understanding of this theory, cancer stem cells are never replaced by other cells that belong to a group beneath stem cells. However, our data poses a question about this point. We believe that cancer cells can change their phenotype under the influence of their growth environment.

The concept of cancer stem cells was initially suggested by a study of leukemia. The existence of leukemia stem cells (LSCs) explained many clinical issues, for example, why leukemic cells can acquire resistance against intensive chemotherapies. Initially, the characteristics of LSCs were considered to resemble those of normal hematopoietic stem cells. However, it has been becoming clear that leukemic cells have high ability of plasticity. This means that leukemic cells, other than LSCs, may occasionally de-differentiate to LSCs and may even differentiate to mesenchymal cells. We are studying this plasticity using JAS-R megakaryocytic leukemia cells, which we established, by changing the culture conditions that mimic the microenvironment of bone marrow.

Molecular pharmacology of anticancer agents

Radiation and chemotherapy are the main choices for comprehensive cancer treatment. In our laboratory, we are investigating the anticancer activity of the following chemicals: telomerase inhibitors, histone deacetylase (HDAC) inhibitors, tyrosine kinase inhibitors, and DNA topoisomerase I (Top1) inhibitors.

We recently found that HDAC inhibitors were suitable agents for combination therapy with radiation and tyrosine kinase inhibitors. The dose of radiation to induce apoptosis was reduced to 20% by simultaneous treatment with HDAC inhibitors. This augmenta-

tion 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 with an ubiquitin kinase.

Top1 inhibitors are key drugs for the treatment of solid tumors, especially colon cancers. We have successfully isolated several clones resistant to SN38 (an active compound of irinotecan) and analyzed their DNA sequences. Three independent mutations of the Top1 gene were found. Each mutation site is closely related to the degree of resistance to Top1 inhibitors in colon cancer cells. To further study the relation between the mutations and resistance, the function of each mutated Top1 gene is being studied using a Top1-defective yeast.

Molecular genetic approach to neurological diseases

Spinal muscular atrophy (SMA) is a degenerative disorder leading to muscular atrophy. A mutation of survival of motor neuron 1 (SMN1) is responsible for the onset of the disease. Unlike other mammals, human beings have SMN2, a member of the same family as SMN1. It remains unclear why intact SMN2 cannot compensate for the function of SMN1 in patients with SMA. SMN2 has several nucleic acid substitutions compared with SMN1. One substitution located in exon 7 seems to play an important role in the disturbance of intact SMN2 production. The RNA-binding heterogeneous nuclear ribonucleoproteins A1 and A2 may be involved in this disturbance through the splicing and translation of SMN2. These findings may contribute to the development of new treatments for SMA.

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 can be difficult to predict at disease onset. 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. We have recently found that a single nucleotide polymorphism of brain-derived neurotrophic factor was useful for predicting frontal lobe functional impairment during disease progression.

Publications

Kawano T, Akiyama M, Agawa-Ohta M, Mikami-Terao Y, Iwase S, Yanagisawa T (Saitama Med Univ), Ida H, Agata N, Yamada H. Histone deacetylase inhibitors valproic acid and depsipeptide sensitize retinoblastoma cells to radiotherapy by increasing H2AX phosphorylation and p53 acetylation-phosphorylation. Int J Oncol 2010; 37: 787-95.

Akiyama M, Kawano T, Mikami-Terao Y, Agawa-Ohta M, Yamada O (Tokyo Women's Med Coll), Ida H, Yamada H. Erythropoietin activates telomerase through transcriptional and posttranscriptional regulation in human erythroleukemic JAS-REN-A cells. Leuk Res 2011; 35: 416-8.

Ahmad R, Rajabi H, Kosugi M, Joshi MD, Alam M, Vasir B, Kawano T, Khabanda S, Kufe D. MUC1-C oncoprotein promotes STAT3 activation in an autoinductive regulatory loop. *Sci Signal* 2011; **4**: ra9.

Rocha-Sousa A¹, Hayashi T, Gomes NL¹, Penas S¹, Brandão E¹, Rocha P¹, Urashima M, Yamada H, Tsuneoka H, Falcão-Reis F¹ (¹Hosp São João). A novel mutation (Cys83 Tyr) in the second zinc finger of NR2E3 in enhanced S-cone syndrome. Graefes Arch Clin Exp Ophthalmol 2011; **249:** 201–8.

Institute of DNA Medicine Department of Molecular Immunology

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

General Summary

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

Research Activities

Pleiotropic function of interleukin-31

Interleukin (IL)-31 is a T-cell-derived cyotokine that induces severe pruritus, hair loss, and dermatitis and is involved in allergic diseases, such as atopic dermatitis and bronchitis. To investigate the function of IL-31, IL-31 transgenic (Tg) mice were generated in our laboratory. In addition to scratching behavior and hair loss as reported previously, enhancement of serum immunoglobulin (Ig) E level was observed in the IL-31 Tg mice. Furthermore, these pleiotropic functions were verified by the administration of IL-31 into normal mice. To further analyze the mechanism of IgE production by IL-31, we are seeking factors enhancing T helper type 2 (Th2) cytokine production, focusing on the IL-31 receptor-expressing cells, such as keratinocytes, macrophages, and granulocytes.

A rice-based edible vaccine expressing Japanese cedar pollen allergens induces oral tolerance in Japanese monkeys with Japanese cedar pollinosis

Japanese cedar (*Cryptomeria japonica*: CJ) pollinosis affects more than 30% of the Japanese population and is, thus, one of the most common diseases in Japan. Furthermore, CJ pollinosis has been found to occur naturally in Japanese monkeys (*Macaca fuscata*), which show symptoms similar to those of human patients.

Plants have recently been recognized as a form of bioreactor for the cost-effective production of large-scale recombinant proteins. The edible tissue of plants further provides the significant benefit of achieving a simple method of mucosal delivery of vaccines without the need for complicated purification steps.

Our previous study showed that oral administration of Tg rice seeds that have accumulated high concentrations of polypeptides derived from CJ pollen allergens to mice reduces their serum IgE levels and T-cell proliferative responses to CJ allergens, proving the efficacy of oral immunotherapy for the treatment of pollinosis.

In this study, the Tg rice plants that had accumulated high concentrations of JC allergens were used for oral immunotherapy for CJ pollinosis of monkeys. Five monkeys with CJ pollinosis were fed once a day with 20 g of the rice seeds containing about 50 to 60 mg of

allergens for 3 months. No side effects, such as urticaria, dyspnea, vomiting, and weight loss, were observed during immunotherapy. One and a half months after the start of feeding, proliferative responses of T cells to JC allergens in 4 of 5 monkeys were significantly inhibited compared with those in monkeys at the start of feeding. However, their T-cell responses to CJ allergens were restored 1 month after the end of feeding.

These results indicate that oral immunotherapy with Tg rice seeds is safe and effective for the treatment of pollinosis.

Construction of a new anticancer strategy focused on glycosylation

We are developing a novel anticancer strategy that induces cytotoxic T cells against nonpolarized cells represented by cancer cells, by enhancing MHC class I-restricted antigen presentation by inhibiting N-glycosylation.

Analysis of the N-glycosylation structure that controls the secretion of IL-31 showed that some structures of N-glycosylation were able to enhance MHC class I-restricted antigen presentation. On the basis of this finding, we are developing a new vaccine that induces cytotoxic T cells against cancer or viruses with artificial immature N-glycosylated proteins.

Publications

Fukuda T, Akiyama N, Ikegami M, Takahashi H, Sasaki A, Oka H, Komori T, Tanaka Y, Nakazato Y, Akimoto J, Tanaka M, Okada Y, Saito S. Expression of hydroxyindole-o-methyltransferase enzyme in the human central nervous system and in pineal parenchymal cell tumors. J Neuropathol Exp Neurol 2010; 69: 498-510. Kurosaka D, Hirai K, Nishioka M, Miyamoto Y, Yoshida K, Noda K, Ukichi T, Yanagimachi M, Furuya K, Takahashi E, Kingetsu I, Fukuda K, Yamada A. Clinical significance of serum levels of vascular endothelial growth factor, angiopoietin-1, and angiopoietin-2 in patients with rheumatoid arthritis. *J Rheumatol* 2010; **37**: 1121-8.

Yoshida K, Kurosaka D, Joh K, Matsushima S, Takahashi E, Hirai K, Noda K, Ukichi T, Furuya K, Yanagimachi M, Kingetsu I, Fukuda K, Yamada A. Fasciitis as a common lesion of dermatomyositis, demonstrated early after disease onset by en bloc biopsy combined with magnetic resonance imaging. Arthritis Rheum 2010; 62: 3751–9.

Institute of DNA Medicine Department of Molecular Cell Biology

Yoshinobu Manome, Professor

General Summary

Our research goal is to analyze the molecular events of cells under physiological and pathological conditions. To achieve this goal, we use morphological and biochemical approaches combined with nucleic acid modification. We have used transfection of naked DNA or short interfering RNA to modulate protein expression and have used fluorescent nanoparticle conjugates to visualize or quantify target molecules. By combining methods of molecular and cellular biology, we are exploring medical life sciences.

Research Activities

Development of nucleic acids delivery into malignant glioma cells by acoustic energy Malignant glioma is an intractable disease of the human brain. Although many adjuvant therapies, such as radiotherapy, chemotherapy, and immunotherapy, have been developed, the prognoses of patients remain unsatisfactory. Therefore, we are exploring alternative therapies, such as sonodynamic therapy. A characteristic of the malignant glioma is that in spite of the poor prognosis, distant metastasis seldom occurs and death is due mostly to local recurrence. In this situation, we developed a theragnosis system, which combines therapy and diagnosis, for glioma. The system enables radiotherapeutic ultrasound to be applied to a glioma as a local treatment while the process is monitored. This year, we attempted to develop another combination of nucleic acid delivery to the theragnosis system. We selected several intracellular signal molecules as targets. First, we found that in most glioma cell lines, Rho kinase isoforms (ROCK1 and ROCK2), epidermal growth factor receptor (EGFR), and signal transducer and activator of transcription (STAT) 3 were expressed and phosphatase and tensin homologue (PTEN) was down-regulated. Next we found that transduction of short hairpin RNA (shRNA) to ROCK1, ROCK2, EGFR and STAT3 down-regulated the expressions of respective molecules, and forced expression of PTEN inhibited cell proliferation. We also found that down-regulation of ROCK1 and expression of PTEN prolonged the G2 phase of the cell cvcle and increased sensitivity to alkylating agents. Because these molecules are potential therapeutic targets, acoustic conditions for delivery were evaluated.

Biochemical application of thyroid carcinoma specific antibody

Diagnosis of thyroid cancer involves such methods as palpation, ultrasonography, fineneedle aspiration cytology, scintigraphy, and blood tests. To increase diagnostic precision and to alleviate the burdens on both patients and physicians, we have developed detection systems with a thyroid carcinoma-specific antibody (JT95) and fluorescent nanoparticles (quantum dots). The direct binding of quantum dots and JT95 monoclonal
antibodies was applicable to Western blotting analysis, an enzyme-linked immunosorbent assay-like system, and fluorescent microscopic analysis of the SW1736 thyroid carcinoma cell line. These easily handled techniques may increase diagnostic precision.

Function of proopiomelanocortin in cardiomyocytes

The expression of proopiomelanocortin (POMC) in HL-1 cardiomyocytes was studied. An overexpression vector plasmid of POMC was constructed and will be used to study the release of ACTH from HL-1 cardiomyocytes overexpressing POMC. In addition, the effects of Ca^{2+} channel blockers on the expression of steroidogenic enzymes were investigated using NCI-H295R adrenocortical carcinoma cells.

Publications

Inaba N, Ishizawa S, Kimura M, Fujioka K, Watanabe M, Shibasaki T, Manome Y. Effect of inhibition of the ROCK isoform on RT2 malignant glioma cells. Anticancer Res 2010; **30**: 3509-14. Hoshino A, Iimura T, Ueha S, Hanada S, Maruoka Y, Mayahara M, Suzuki K, Imai T, Ito M, Manome Y, Yasuhara M, Kirino T, Yamaguchi A, Matsushima K, Yamamoto K. Deficiency of chemokine receptor CCR1 causes osteopenia due to impaired functions of osteoclasts and osteoblasts. J Biol Chem 2010; **285**: 28826-37. Watanabe M, Fujioka K, Akiyama N, Takeyama H, Manabe N¹, Yamamoto K¹, Manome Y ('Natl Ctr Global Health Med). Conjugation of quantum dots and JT95 IgM monoclonal antibody for thyroid carcinoma without abolishing the specificity and activity of the antibody. *IEEE Trans Nanobioscience* 2011; **10**: 30-5.

Shirasu M, Fujioka K, Kakishima S, Nagai S, Tomizawa Y, Tsukaya H, Murata J, Manome Y, Touhara K. Chemical identity of a rotting animallike odor emitted from the inflorescence of the titan arum (Amorphophallus titanum). Biosci Biotechnol Biochem 2010; 74: 2550-4.

Fujioka K, Tomizawa Y, Yamamoto K, Manome Y. Numerical conversion of smell from frozen food and its variation with temperature (in Japanese). *Nihon Aji to Nioi Gakkaishi* 2010; **17:** 533-5.

Institute of DNA Medicine Project Laboratory for Kidney Regeneration

Takashi Yokoo, Assistant Professor and Director

General Summary

Recently, much attention has been focused on the use of embryonic organs as a source for xenotransplantation. This strategy involves transplantation of the embryonic organ before vascularization, with the subsequent development of a mature organ in the recipient. Because the vasculature of the transplant is of host origin and the embryonic organ per se is less immunogenic, intense immunomodulation should not be required. One study has found that a pig metanephros was able to grow and differentiate into mature renal tissue in the rat omentum; the transplanted tissue produced urine and, after intact ureteroureterostomy, anephric rats started to void and showed prolonged lifespans. Previously, we successfully rebuilt metanephroi from human mesenchymal stem cells (hMSCs) using a program of nephrogenesis in a growing xenoembryo. Combining this success with the concept of embryonic organ transplantation should result in the regeneration of an autologous self-kidney. To this end, we transplanted metanephroi derived from hMSCs into rat omentum and were able to establish mature xenokidneys. We demonstrated that the vasculature of the transplant is of host origin and is capable of producing urine by filtration of the host's blood. Furthermore, the transplant expresses xenoerythropoietin, the production of which is regulated by the degree of anemia in the host. Our ultimate goal is to establish an entire functional kidney in patients with endstage renal disease; however, because the kidney has various other functions in addition to urine production, we believe that each function should be reestablished using separate systems that may be subsequently unified. In previous studies, we transplanted tissue to the omentum; this site is used mainly because it is not confined by a tight capsule and is easily accessed with endoscopy. However, the optimal site for transplantation of each of the renal functions should be determined separately on the basis of anatomical and physiological considerations.

Therefore, this year, we investigated the effect of the transplantation site on the production of renin and erythropoietin as indicators of renal function.

Research Activities

A xenotransplanted metanephros may undergo complete nephrogenesis in the host animal, forming a functional kidney. This suggests that, in the future, xenometanephroi could become an unlimited source of material for renal transplantation. Although the omentum is the primary site for transplantation, we speculate that the growth of the transplant could differ depending on the site of transplantation. Thus, we determined the optimal transplantation site for the metanephros to retain its ability to produce renin and erythropoietin. Rat metanephroi were transplanted into the omentum, the paraaortic area, or both the omentum and the paraaortic area of unilaterally nephrectomized host rats. After 2 to 3 weeks, blood was rapidly withdrawn to induce production of renin and erythropoietin in the transplants. Histological analysis indicated that transplants in both the paraaortic area and the omentum were well differentiated, demonstrating polarity of the medulla through to the cortex. Plasma renin activity increased in response to the induction procedure, but transplants in the paraaortic area expressed plasma renin activity more effectively than did those in the omentum. Real-time polymerase chain reaction revealed higher levels of renin messenger RNA expression in transplants in the paraaortic area than in transplants in the omentum. Although erythropoietin production increased 24 hours after the induction procedure, the levels did not differ significantly between transplantation to the omentum, transplantation to the paraaortic area. Compared with transplantation to the omentum, transplantation to the paraaortic area results in better renin production, whereas the transplantation site does not affect erythropoietin production.

Publications

Gheisari Y, Yokoo T, Matsumoto K, Fukui A, Sugimoto N, Ohashi T, Kawamura T, Hosoya T, Kobayashi E. A thermoreversible polymer mediates controlled release of glial cell line-derived neurotrophic factor to enhance kidney regeneration. *Artif Organs* 2010; **34:** 642-7.

Reviews and Books

Yokoo T, Yanagita M. Stem cell therapy against oxidative stress and hypoxia. In: Miyata T, Eckardt KU, Nangaku M, editors. Studies in renal disorders. New York: Springer; 2011. p. 673-88.

Department of Neuroscience Division of Neuropathology

Satoshi Kurihara, Professor and Director Junko Fujigasaki, Assistant Professor Takahiro Fukuda, Assistant Professor

General Summary

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

Research Activities

Pathophysiological study of polyubiquitination in lysosomal diseases

Objective: This study investigated the pathophysiology of polyubiquitination in lysosomal diseases.

Material and methods: We analyzed skeletal muscle and central nervous system (CNS) neurons from model mice of Pompe disease, prosaposin deficiency, Niemann-Pick disease type C, Fabry disease, Hunter disease, and Sly disease and human patients with Pompe disease and Gaucher disease by means of immunohistochemical methods and antibodies against ubiquitin, K48 polyubiquitin, K63 polyubiquitin, p62, microtubule-associated protein light chain 3 (LC3), and lysosome-associated membrane protein 2 (LAMP2).

Results: Skeletal muscle from patients with Pompe disease contained aggregates immunoreactive for p62, ubiquitin, K48 polyubiquitin, K63 polyubiquitin, and LC3. Neurons from the CNS of prosaposin deficiency and Niemann-Pick disease type C (mice and humans) had small numbers of aggregates immunoreactive for p62, ubiquitin, K48 polyubiquitin, K63 polyubiquitin, and LC3. In mice models of Fabry disease, Hunter disease, and Sly disease and in human patients with Fabry disease and Gaucher disease, the CNS contained LAMP2-immunoreactive lysosomes with vacuolation. There were, however, few aggregates immunoreactive for p62, LC3, or ubiquitin.

Discussion: We confirmed that polyubiquitination involving the ubiquitin proteolysis system and the autophagy lysosome system would function in Pompe disease, prosaposin deficiency disease, and Niemann-Pick disease C.

Histopathological analysis of rat peripheral nerves with an in vivo cryotechnique

Experimental animals are used to examine the pathogenesis of various disorders of peripheral nerves. However, observing the fine structure of the peripheral nerves can be difficult with conventional histopathological analysis of paraformaldehyde-fixed, paraffinembedded specimens. We attempted to examine the peripheral nerves of experimental rats with an *in vivo* cryotechnique. Sciatic nerves of rats were rapidly frozen by pouring isopentane-propane cryogen over them. The sciatic nerves were placed in a freeze-sub-

stitution solution (absolute acetone containing 2% paraformaldehyde), and the temperature of the sciatic nerves was gradually returned to room temperature. After being washed, the nerves were transferred to chloroform and embedded in paraffin. Sections of the paraffin-embedded samples were used for hematoxylin and eosin, Bodian, and Klüver-Barrera staining and immunohistochemical analysis. The axons were preserved from shrinkage, and the fine structure of the examined nerves could be observed. The structure of the nodes of Ranvier could be visualized with this preparation. Schmidt-Lanterman incisures were visible with immunostaining for E-cadherin or β catenin. Preparation of specimens with our *in vivo* cryotechnique provided good resolution for microscopic examination of the peripheral nerves.

Publications

Kyosen SO, lizuka S, Kobayashi H, Kimura T, Fukuda T, Shen J, Shimada Y, Ida H, Eto Y, Ohashi T. Neonatal gene transfer using lentiviral vector for murine Pompe disease: long-term expression and glycogen reduction. *Gene Ther* 2010; **17:** 521-30.

Fukuda T, Akiyama N, Ikegami M, Takahashi H, Sasaki A, Oka H, Komori T, Tanaka Y, Nakazato Y, Akimoto J, Tanaka M, Okada Y, Saito S. Expression of hydroxyindole-O-methyltransferase enzyme in the human central nervous system and in pineal parenchymal cell tumors. J Neuropathol Exp Neurol 2010; 69: 498-510.

Kobayashi H, Takahashi-Fujigasaki J, Fukuda

T, *Sakurai K*, *Shimada Y*, *Nomura K*, *Ariga M*, *Ohashi T*, *Eto Y*, *Otomo T*, *Sakai N*, *Ida H*. Pathology of the first autopsy case diagnosed as mucolipidosis type III alpha/beta suggesting autophagic dysfunction. *Mol Genet Metab* 2011; **102:** 170-5.

Takahashi-Fujigasaki J, Breidert T¹, Fujigasaki H², Duyckaerts C¹, Camonis JH¹, Brice A¹, Lebre AS¹ (INSERM U781, ²Musashino Red Cross Hosp). Amyloid precursor-like protein 2 cleavage contributes to neuronal intranuclear inclusions and cytotoxicity in spinocerebellar ataxia-7 (SCA7). Neurobiol Dis 2011; **41**: 33-42.

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 mainly through intercommunication via nervous systems. To understand how activities of the organs affect brain activity and, in turn, how the brain controls the activities of 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 of the central nervous system "wiring," realized through variability of synaptic connections in response to various environmental changes, form the core mechanism for optimizing human and animal behaviors. We use approaches at the molecular, cellular, and network levels, including the patch-clamp recording of synaptic currents 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 structural consolidation is involved in synaptic potentiation at the excitatory synapses between afferent fibers arising from the lateral parabrachial nucleus and neurons in the central nucleus of the amygdala, an important structure in the expression of emotional behavior. We also demonstrated using neonatal capsaicin treatment that peripheral C fibers expressing the transient receptor potential vanilloid 1 channel are necessary for establishing this synaptic potentiation. This result indicates that a specific set of nociceptive afferents is essential for the pathological enforcement of the link between nociception and negative emotion.

Synaptic mechanism underlying acquisition and extinction of fear memory

The Pavlovian fear-conditioning paradigm depends on the association between a contiguously applied cue (e.g., tone) and an aversive signal (e.g., electrical shock). We aimed to establish transgenic mice that express specific fluorescent marker proteins in response to fear conditioning or its extinction to enable selective fluorescence-guided recording of the identified amygdala neurons in brain slices after behavioral tests. This preparation will enable analyses of specific synaptic changes in the neurons involved in these processes.

Glia-neuron interaction at central synapses

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

Specific mechanism underlying motor neuron vulnerability

We have previously demonstrated in brainstem slices that anoxia and hypoxia facilitate glycine release in an action potential-independent manner in hypoglossal motor neurons but not in the dorsal nucleus of the vagus nerve. To determine whether this facilitation is a common feature in cranial motor neurons, we recorded synaptic inputs in facial and oculomotor neurons and analyzed how these inputs were affected by anoxia. We found that in oculomotor neurons, the anoxia facilitated release of GABA but not of glycine. This difference in the response to anoxia of the inhibitory transmission between distinct motor neurons might provide a basis for the distinct vulnerability of these motor neurons in the motor neuron degenerative diseases.

Publications

Yamamoto K, Noguchi J, Yamada C, Watabe AM, Kato F. Distinct target cell-dependent forms of short-term plasticity of the central visceral afferent synapses of the rat. *BMC Neurosci* 2010; **11:** 134.

Moody TD, Watabe AM, Indersmitten T, Komiyama NH, Grant SG, O'Dell TJ. Beta-adrenergic receptor activation rescues theta frequency stimulation-induced LTP deficits in mice expressing C-terminally truncated NMDA receptor GluN2A subunits. *Learn Mem* 2011; **18:** 118-27.

Ono K, Tsukamoto-Yasui M, Hara-Kimura Y, Inoue N, Nogusa Y, Okabe Y, Nagashima K, Kato F. Intragastric administration of capsiate, a transient receptor potential channel agonist, triggers thermogenic sympathetic responses. J Appl Physiol 2011; **110**: 789-98.

Institute for High Dimensional Medical Imaging

Naoki Suzuki, Professor

Asaki Hattori, Associate Professor

General Summary

The goal of our research is to develop new imaging systems that can be applied to clinical medicine now and in the future. High-dimensional, i.e., 3-dimensional (3D) and 4-dimensional (4D), imaging techniques have enabled noninvasive, realistic, uninhibited, and accurate observations of human spatial structures and their dynamics. The availability of real-time imaging using high-performance computers and medical virtual reality systems has expanded the possibilities for diagnosis, treatment, surgery, and medical education. The Institute for High Dimensional Medical Imaging has, therefore, established a system that facilitates cooperative research and development with international researchers and organizations.

Research Activities

Clinical application of high-definition, real-time medical imaging

We are performing research on the development of medical high-definition imaging technology and its clinical application using functional and morphological data obtained with X-ray computed tomography (CT) and magnetic resonance imaging.

This year, we developed a system that can evaluate a physician's diagnosis and treatment of a patient with medial compartment osteoarthritis of varus knee. The system can analyze the trajectory of a patient's knee adduction moment and the center of pressure on the rear foot during walking.

This research is being performed by departments of our university in collaboration with Osaka University, Tsurumi University, and Mayo Clinic (Rochester, MN, USA).

Development of an endoscopic surgical robot system

We are developing an endoscopic surgical robot system that can be used to perform natural orifice translumenal endoscopic surgery and single-port surgery. Robotic instruments enter the abdominal cavity orally or through a single incision in the navel and are used to perform surgery on the abdominal organs. Last year, we improved the robot arms so that they could have more freedom of movement. This year we improved them so that they could conduct more complicated tasks. We also isolated the movements of the camera, which are the eyes of the robot, so that the robot's upper limb can perform movements closer to that of the humans.

This research is being performed in collaboration with Kyushu University's Department of Surgery.

Development of a simulator for the endoscopic surgical robot system

To perform surgery with the surgical robot system described above, the operator requires

training because the operative method differs greatly from that of conventional surgery. Therefore, we are developing a simulator system for animal experiments that has the same functions as the actual surgical robot system. This year, we developed a basic training system to control the robot arms for beginner operators who are not familiar with using the robot by setting up tasks in a virtual reality environment. The tasks consist of stages of different levels. We also improved the training system for using organ models by enabling the system to record and save all processes during training. After the training, the operator can observe and analyze the changes of the operational field during training in 4D from any viewpoint.

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 which 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. This year we also developed a new pointer-based navigation system. Combining the use of this new navigation system with the endoscope-based navigation system, the operator can grasp the 3D structure of the operational field more intuitively. For the navigation system under development in collaboration with the Department of Surgery, we have developed a system that uses a laparoscope with an appropriate shape for capturing images of the operative field during laparotomy. We plan to apply this system clinically in the near future.

Application of high-definition medical image analysis to forensic medicine

By applying technology that we have developed for analyzing high-definition medical images, we are analyzing X-ray CT data sets of crime victims with the aim of developing new methods for future criminal investigations and for establishing new methods for creating court documents. This year, we have improved the system to allow better 3D analysis of a victim's injuries. With this system, we have analyzed the position, depth, and angle of a victim's wounds in 3D using X-ray CT data sets from cases of murder and attempted murder at the request of the Tokyo District Prosecutor's Office.

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

Ogawa T^I, Ikawa T^I, Shigeta Y^I, Kasama S^I, Ando E^I, Fukushima S^I, Hattori A, Suzuki N ('Tsurumi Univ). Virtual reality image applications for treatment planning in prosthodontic dentistry. Stud Health Technol Inform 2011; **163**: 422-4.

Ikawa T¹, Ogawa T¹, Shigeta Y¹, Kasama S¹,

Hirabayashi R¹, Fukushima S¹, Hattori A, Suzuki N (¹Tsurumi Univ). Design for functional occlusal surface of CAD/CAM crown using VR articulator. *Stud Health Technol Inform* 2011; 163: 239-41.

Kasama S¹, Ogawa T¹, Ikawa T¹, Shigeta Y¹, Hirai S¹, Fukushima S¹, Hattori A, Suzuki N ('Tsurumi Univ). Influence of metal artifacts on the creation of individual 3D cranio-mandibular models. Stud Health Technol Inform 2011; 163: 261-3.

Yamazaki Y^I, Ogawa T^I, Shigeta Y^I, Ikawa T^I, Kasama S^I, Hattori A, Suzuki N, Yamamoto T^I, Ozawa T^I, Arai T^I (^ITsurumi Univ). Clinical performance of dental fiberscope image guided system for endodontic treatment. *Stud Health Technol Inform* 2011; **163**: 713-5.

Kawakami H (Osaka Police Hosp), Sugano N (Osaka Univ), Miki H (Osaka Natl Hosp), Yonenobu K (Osaka-Minami Natl Hosp), Hattori A, Suzuki N. Development of the 4D gait analysis system with the locus of grounding point on the behind foot (in Japanese). Nihon Computer Geka Gakkaishi 2010; **12:** 527-31.

Suzuki N, Hattori A, Tanoue K¹, leiri S¹, Konishi K¹, Tomikawa M¹, Kenmotsu H¹, Hashizume M¹ (^IKyushu Univ). Scorpion shaped endoscopic surgical robot for NOTES and SPS with augmented reality functions. Lecture Notes in Computer Science 2010; **6326:** 541-50.

Institute of Clinical Medicine and Research

Norio Tada, Professor and Director

Akihito Tsubota, Associate Professor

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

General Summary

The aim of our research is to fill the gap between clinical medicine and fundamental medicine and to shorten the time needed for discoveries to go from bench to bedside. We are proud to report that during the last few years we have made good progress in the development of a drug delivery system using nanotechnology. We also made progress in gene technology, especially in the treatment of hepatitis C virus (HCV) infection and liver cancer. In the field of lipid metabolism related to atherosclerosis, we succeeded in measuring lipoprotein A, a atherosclerotic lipoprotein, with our newly developed ion-exchange chromatography.

Research Activities

Functional analysis of oxidative stress-induced liver carcinogenetic genes

Our comprehensive gene expression profiling analysis has identified candidate genes associated with oxidative stress-induced liver carcinogenesis in an animal model with naturally occurring hepatotumorigenesis. We are now analyzing the function of the gene signatures in the process of liver carcinogenesis and the mechanism of tumorigenesis.

Transporter gene in the treatment of chronic HCV infection

Pegylated interferon plus ribavirin combination therapy is the standard of care (SOC) treatment for chronic HCV infection. In the SOC treatment, exposure of HCV to ribavirin in hepatocytes is critical for virus eradication. Ribavirin is transported into hepatocytes by cell membrane transporters. We are investigating the function of transporters and the association of single nucleotide polymorphisms of the gene with treatment response.

Comprehensive gene expression profiling analysis of microRNA/messenger RNA in liver tissue

We are profiling and analyzing the expression of microRNA/messenger (m) RNA in the liver tissue of patients with chronic HCV infection who would receive SOC treatment. We are analyzing whether the microRNA/mRNA candidates can be associated with treatment response in chronic HCV infection. When the candidates affect the treatment outcome, the function of the microRNA/mRNA will be investigated in detail.

The temporal and spatial manipulation of "basket-type organic/inorganic-hybrid structure" as a future theranostic nanomedicine

Free manipulation of the movement of drugs with remote controlled light/magnetism/ ultrasound used in cutting-edge medical technology is expected to be a next-generation technology. Remotely manipulating the speed and position of nanoparticles, which are mineral capsules that respond to various types of physical energy and are filled with organic drugs, will lead to an innovative technology that allows "pinpoint and perfectly timed" diagnosis and treatment.

We aim for the realization of innovative nanomedicine in which we can remotely control the accumulation, release, and effects of drugs by using nanosized capsules that efficiently convert light, magnetic, and ultrasonic energies. This is unprecedented research in which we can apply Japan's world-leading nanotechnology to medicine. It will allow highly sensitive, rapid diagnosis and highly effective treatment that is gentle to the body for incurable diseases and for diseases that are difficult to diagnose. The realization of medical care that is gentle to the weak, such as elderly persons, will contribute to the promotion of a long and healthy life, reduced healthcare costs, and the development of the healthcare industry. Moreover, because this technology can precisely control the behavior of drugs, it can be applied to wide areas, such as pharmacology and biotechnology.

Studies of lipid metabolism and atherosclerosis

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

Publications

Tsubota A, Fujise K, Namiki Y, Tada N. Peginterferon and ribavirin treatment for hepatitis C virus infection. *World J Gastroenterol* 2011; **17:** 419-32.

Aizawa M, Tsubota A, Fujise K, Sato K, Baba M, Takamatsu M, Namiki Y, Ohkusa T, Tajiri H. Overlap/switch to adefovir monotherapy for lamivudine-resistant patients who responded to combination therapy: a pilot controlled study. *Intern Med* 2010; **49:** 1067-72.

Fuchigami T¹, Kawamura R¹, Kitamoto Y¹,

Nakagawa M², Namiki Y (¹Tokyo Inst Tech, ²Tohoku Univ). Ferromagnetic FePt-nanoparticles/polycation hybrid capsules designed for a magnetically guided drug delivery system. Langmuir 2011; **27**: 2923-8. Epub 2011 Feb 3. Yoshida H, Shimizu M, Ikewaki K, Taniguchi I, Tada N, Yoshimura M, Rosano G, Dahlof B, Mochizuki S; Jikei Heart Study group. Sex differences in effects of valsartan administration on cardiovascular outcomes in hypertensive patients: findings from the Jikei Heart Study. J Hypertens 2010; 28: 1150-7.

Yoshida H, Ishikawa T, Suto M, Kurosawa H, Hirowatari Y, Ito K, Yanai H, Tada N, Suzuki M. Effects of supervised aerobic exercise training on serum adiponectin and parameters of lipid and glucose metabolism in subjects with moderate dyslipidemia. J Atheroscler Thromb 2010; **17:** 1160-6.

Yanai H, Kaneko H, Yoshida H, Tada N. A significant association between impaired glucose metabolism and polymyalgia rheumatica. *J Atheroscler Thromb* 2010; **17:** 1108-9.

Yanai H, Tomono Y, Yoshida H, Tada N. Therapeutic application of diacylglycerol oil for the metabolic syndrome (in Japanese). *Rinsho Byori* 2010; **58:** 39-44.

Hirowatari Y, Yoshida H, Kurosawa H, Shimura Y, Yanai H, Tada N. Analysis of cholesterol levels in lipoprotein(a) with anion-exchange chromatography. *J Lipid Res* 2010; **51:** 1237-43.

Koido S, Homma S, Hara E, Namiki Y, Takahara A, Komita H, Nagasaki E, Ito M, Ohkusa T, Gong J, Tajiri H. Regulation of tumor immunity by tumor/dendritic cell fusions. *Clin Dev Immunol* 2010; **2010**: 516768.

Fujise K, Tatsuzawa K, Kono M, Hoshina S, Tsubota A, Niiya M, Namiki Y, Tada N, Tajiri H. A mutation of the start codon in the X region of hepatitis B virusDNA in a patient with non-B, non-C chronic hepatitis. *World J Hepatol* 2011; **3**: 56-60.

Sasaki T, Hiki Y, Nagumo S, Ikeda R, Kimura H, Yamashiro K, Gojo A, Saito T, Tomita Y, Utsunomiya K. Acute onset of rheumatoid arthritis associated with administration of a dipeptidyl peptidase-4 (DPP-4) inhibitor to patients with diabetes mellitus. Diabetol Int 2010; 1: 90-2.

Medical Engineering Laboratory

Hiroshi Furuhata, Professor

Masayuki Yokoyama, Associate Professor and Director

General Summary

Medical engineering is an essential base for developments in medicine. In our laboratory, there are 2 key technologies: ultrasound and polymeric biomaterials. We have developed ultrasound technology for a new thrombolytic treatment for acute ischemic stroke. Our ultrasound research uses medium-frequency ultrasound and close collaboration with clinical departments and basic science departments both in our university and in other universities and hospitals. For the other key technology, polymeric biomaterials, we have been researching their applications to drug delivery systems. We have also recently applied polymeric biomaterials to diagnostic imaging through the synthesis of new polymeric contrast agents. In particular, we are studying polymeric micelle systems that can deliver both drugs and contrast agents. Therefore, these systems are called "theranostic" systems because they have functions in both therapy and diagnostics. Additionally, we have research topics combining the ultrasound and polymericbiomaterials technologies. One example is ultrasound-assisted targeting of polymeric drug carrier systems.

Research Activities

Medical application of ultrasound

We have applied ultrasound to transcranial therapy for ischemic stroke. Although injection of tissue plasminogen activator has been the only effective therapy, an even more effective therapy is urgently required. The transcranial ultrasound can enhance thrombolytic activity of tissue plasminogen activator. Our technology features the use of medium-frequency ultrasound, which possesses greater thrombolytic enhancement than does ordinary ultrasound. However, the medium-frequency ultrasound is believed to be associated with a high risk of brain hemorrhage. To decrease this risk, we control both the period and interval of irradiation and have successfully proven the safety of ultrasound irradiation in models of hypertensive brain ischemia. We have been developing this new therapy through the Super Special Consortium for Supporting the Development of Cutting-Edge Medical Care program supported by the Ministry of Health, Labour and Welfare of Japan.

Polymeric micelle drug carrier systems

Polymeric micelles are assemblies of synthetic polymers that have been applied to drug targeting. Associate Professor Yokoyama, director of this laboratory, is an international pioneer in the development of polymeric micelle targeting systems. Currently, 4 formu-

lations of polymeric micelle anticancer drugs are undergoing clinical trials in Japan, Europe, and the United States. We are trying to establish next-generation science technology in the polymeric micelle systems. We are studying the immunological properties of polymeric micelles. Curiously, the polymeric micelle carriers differ greatly from liposome systems. Although both carrier systems possess poly(ethylene glycol) on their surfaces, only liposomes exhibit an immunological response called the accelerated blood clearance phenomenon. This difference is a great advantage of the polymeric micelle systems. We also study basic chemistry to analyze the drug-incorporated inner core through the use of the Super Photon Ring 8 GeV (SPring 8) Large-Scale Synchrotron Radiation Facility and to prepare polymeric micelles with cross-linked inner cores through photochemistry. These cross-linked micelles are extremely useful for clarifying the in vivo fates of polymeric micelles.

Polymer-based contrast agents for image diagnosis

We have developed new polymeric micelle contrast agents for magnetic resonance (MR) imaging. These agents were shown to target solid tumor sites and to exhibit clear MR images of extremely small tumors. Therefore, the polymeric micelles can be used for tumor theranostics because they can direct both drugs and contrast agents towards solid tumors. Furthermore, we are studying a novel application of the polymeric carrier system for diagnosis of ischemic stroke. We observed that the polymeric micelle MR contrast agent successfully targets a specific site in the ischemic hemisphere and provides high-contrast images that were not obtained with a conventional low-molecular-weight MR contrast agent. Therefore, the polymeric micelle carrier system may be extremely useful for both the diagnosis and treatment of ischemic stroke.

Publications

Azuma T¹, Ogihara M², Kubota J², Sasaki A², Umemura S³, Furuhata H (¹Hitachi Ctrl Res, ²Hitachi Med, ³Tohoku Univ). Dual-frequency ultrasound imaging and therapeutic bilaminar array using frequency selective isolation layer. *IEEE Trans Ultrason Ferroelectr Freq Control* 2010; **57**: 1211-24.

Hori K¹, Nishihara M², Shiraishi K², Yokoyama M (¹Tohoku Univ, ²Kanagawa Acad Sci Tech). The Combretastatin derivative Cderiv, a vascular disrupting agent, enables polymeric nanomicelles to accumulate in microtumors. *J Pharm Sci* 2010; **99:** 2914–25.

Akiba I¹, Terada N¹, Hashida S¹, Sakurai K¹, Sato T², Shiraishi K, Yokoyama M, Masunaga H², Ogawa H³, Ito K⁴, Yagi N³ (¹Univ Kitakyushu, ²Kanagawa Acad Sci Tech, ³Jpn Synch Radi Res Inst, ⁴RIKEN). Encapsulation of a hydrophobic drug into a polymer-micelle core explored with synchrotron SAXS. Langmuir 2010; **26**: 7544-51.

Shiraishi K, Kawano K¹, Maitani Y¹, Yokoyama M (¹Hoshi Univ). Polyion complex micelle MRI contrast agents from poly(ethylene glycol)-b-poly(l-

lysine) block copolymers having Gd-DOTA; preparations and their control of T(1)-relaxivities and blood circulation characteristics. *J Contr Rel* 2010; **148:** 160-7.

Harada Y¹, Yamamoto T¹, Sakai M¹, Saiki T^{1,2}, Kawano K³, Maitani Y³, Yokoyama M ('Kanagawa Acad Sci Tech, ²Keio Univ, ³Hoshi Univ). Effects of organic solvents on drug incorporation into polymeric carriers and morphological analyses of drug-incorporated polymeric micelles. Int J Pharm 2011; 404: 271-80.

Yokosawa M¹, Sonoda Y¹, Sugiyama S¹, Saito R¹, Yamashita Y¹, Nishihara M², Satoh T², Kumabe T¹, Yokoyama M, Tominaga T¹ ('Tohoku Univ, ²Kanagawa Acad Sci Tech). Convection-enhanced delivery of a synthetic retinoid Am80, loaded into polymeric micelles, prolongs the survival of rats bearing intracranial glioblastoma xenografts. *Tohoku J Exp Med* 2010; **221:** 257-64.

Reviews and Books

Furuhata H. Transcranial sonothrombolysis and

image diagnosis (in Japanese). In: Ishida T, Katsuragawa S, Fujita H, editors. Handbook of medical imaging. Tokyo: Ohmsha; 2010. p. 1158-68. **Yokoyama M.** Molecular imaging for evaluation of drug targeting (in Japanese). *Idenshi Igaku Mook* 2010; **18:** 127-32.

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 design in which detailed data were collected from all cases and in a subcohort representing 5% of all subjects. A full-scale study has been performed with a large sample size of 7,000 patients from 68 institutions.

An administrative office for registration trials was established in the hospital in February 1999, and the system for registration trials in the hospital has been reformed to meet the demands of the new good clinical practice guidelines. Seven clinical research coordinators (CRCs)—6 nurses and 1 pharmacist—now facilitate clinical trials. The CRCs have started to help with both registration trials and investigator-initiated trials. CRCs have been introduced into all registration trials since 2004; the quality and speed of these trials were much improved.

The Ministry of Health, Labour and Welfare started a New 5 Yearly Clinical Trial Action Plan to help registration trials to cope with trials done abroad. This action plan selects 10 core hospitals and 30 major clinical trial institutions. The Jikei University Hospital was named a major clinical trial institution. According to this plan, we reinforced CRCs and introduced a data manager to improve the clinical trial system. We also introduced an information technology system to processing registration trial management. However, because of budgetary problems, this program was ended in our hospital 1 year earlier than scheduled.

Publications

Saito I¹, Suzuki H², Kageyama S, Saruta T¹ ('Keio Univ, ²Saitama Med Sch). Effect of antihypertensive treatment on cardiovascular events in

elderly hypertensive patients: Japan's benidipine research on anti-hypertensive effects in the elderly (J-BRAVE). *Clin Exp Hypertens* 2011; **33**: 13340.

Reviews and Books

Kageyama S. Hypertension in relatively young adults and women with low risks or without organ damages (in Japanese). *Medicina* 2010; **47:** 1144–7.

Kageyama S. Glycaemic control in diabetes complicated with dyslipidaemia and hypertension (in Japanese). *Nihon Iji Shinpo* 2010; **4500:** 50-3.

Kageyama S. Treatment of diabetes in patients complicated with dyslipidaemia and hypertension (in Japanese). *Mebio* 2010; **27(10):** 78-85.

(in Japanese). *Mebio* 2010; **27(10)**: 78-85. *Kageyama S.* Thiazolidinedione derivatives (in Japanese). In: Kageyama S, Kubota K, editors. Yakuzaiekigaku no kiso to jissen. Osaka: Iyaku Janarusha; 2010. p. 61-9.

Kageyama S. Registration trial (in Japanese). In: Kageyama S, Kubota K, editors. Yakuzaiekigaku no kiso to jissen. Osaka: Iyaku Janarusha; 2010. p. 259-64.

Division of Molecular Epidemiology

Mitsuyoshi Urashima, Associate Professor and Director

General Summary

Despite having identical disease diagnoses, some patients may be cured but others may not. This difference cannot be understood with experimental medicine. On the other hand, clinical practice might also not provide the answer. We combined molecular biology and epidemiology to create the Division of Molecular Epidemiology, to clarify the etiology of disease and to predict factors affecting survival.

Research Activities

The Jikei clinical research course

From May 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, 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. Genome and epigenome clinical studies and lead findings

Publications

Suzuki Y, Tamez S, Murakami A, Taira A, Mizuhara A, Horiuchi A, Mihara C, Ako E, Muramatsu H, Okano H, Suenaga H, Jomoto K, Kobayashi J, Takifuji K, Akiyama K, Tahara K, Onishi K, Shimazaki M, Matsumoto M, Ijima M, Murakami M, Nakahori M, Kudo M, Maruyama M, Takahashi M, Washizawa N, Onozawa S, Goshi S, Yamashita S, Ono S, Imazato S, Nishiwaki S, Kitahara S, Endo T, Iiri T, Nagahama T, Hikichi T, Mikami T, Yamamoto T, Ogawa T, Ogawa T, Ohta T, Matsumoto T, Kura T, Kikuchi T, Iwase T, Tsuji T, Nishiguchi Y, Urashima M. Survival of geriatric patients after percutaneous endoscopic gastrostomy in Japan. World J Gastroenterol 2010; 16: 5084-91.

Gorringe KL, George J, Anglesio MS, Ramak-

rishna M, Etemadmoghadam D, Cowin P, Sridhar A, Williams LH, Boyle SE, Yanaihara N, Okamoto A, Urashima M, Smyth GK, Campbell IG, Bowtell DD; Australian Ovarian Cancer Study. Copy number analysis identifies novel interactions between genomic loci in ovarian cancer. *PLoS One* 2010; **5**: e11408.

Rocha-Sousa A, Hayashi T, Gomes NL, Penas S, Brandão E, Rocha P, Urashima M, Yamada H, Tsuneoka H, Falcão-Reis F. A novel mutation (Cys83 Tyr) in the second zinc finger of NR2E3 in enhanced S-cone syndrome. Graefes Arch Clin Exp Ophthalmol 2011; 249: 201-8.

Mezawa H, Sugiura T, Watanabe M, Norizoe C, Takahashi D, Shimojima A, Tamez S, Tsutsumi Y, Yanaga K, Urashima M. Serum vitamin D levels and survival of patients with colorectal cancer: post-hoc analysis of a prospective cohort study. *BMC Cancer* 2010; **10:** 347.

Kimura K, Shimano H, Yokote K, Urashima M, Teramoto T. Effects of pitavastatin (LIVALO tablet) on the estimated glomerular filtration rate (eGFR) in hypercholesterolemic patients with chronic kidney disease. Sub-analysis of the LIVALO Effectiveness and Safety (LIVES) study. J Atheroscler Thromb 2010: **17**: 601-9.

Yokoyama K, Urashima M, Ohkido I, Kono T, Yoshida T, Muramatsu M, Niu T, Hosoya T. L-type voltage-dependent calcium channel alpha subunit 1C is a novel candidate gene associated with secondary hyperparathyroidism: an application of haplotype-based analysis for multiple linked single nucleotide polymorphisms. *Nephron Clin Pract* 2010; **115:** c237-43.

Teramoto T, Shimano H, Yokote K, Urashima M. New evidence on pitavastatin: efficacy and safety in clinical studies. *Expert Opin Pharmacother* 2010; **11:** 817-28.

Shimada T, Urashima M. Ecological study: land cloud cover and suicide in Japan. *Jikeikai Med J* 2010; **57:** 101-11.

Shimada T, Urashima M. Influenza pandemics in Japan during the 20th century. Jikeikai Med J 2010; **57:** 89-99.

Division of Clinical Epidemiology

Masato Matsushima, Associate Professor and Director

General Summary

The Division of Clinical Epidemiology was founded in 2009 as part of the Research Center for Medical Science to promote the activities of clinical research, clinical epidemiology, and education concerning them. Our aim is to support clinicians to solve their own problems in daily practice through epidemiological and clinical research skills.

The research interests of our division are medical communication, evaluation of medical care, behavioral medicine, outcome research, qualitative research, and disease-oriented epidemiological research. In particular, we aim to obtain evidence in the field of primary care, which, despite being the front line of medical practice, suffers from a lack of evidence.

To contribute to undergraduate education, our division holds classes in evidence-based clinical practice to help medical students become skilled physicians able to employ an evidence-based approach.

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

Research Activities

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

This study was, to our knowledge, the first attempt to evaluate the incidence of fever in a Japanese home medical care setting. The subjects were 105 persons (37 men and 68 women) with a mean age (\pm SD) of 82.8 \pm 7.9 years in 1 primary-care clinic. The total number of person-days was 27,546. The incidence of fever >37.2°C was 2.32 per 1,000 person-days (95% confidence interval: 1.75-2.89). Approximately half of the persons receiving home-medical care had fever once a year.

Chronic care model

The chronic care model was developed during the 1990s in the United States to improve the care of chronic illnesses by refining the care-provider system, especially in a primarycare setting. The aim of this research is to clarify the usefulness of the chronic care model in Japan.

The plan consists of 3 steps. The first is to make an official Japanese version of the assessment form "Assessment of Chronic Illness Care" by following World Health Orga-

nization procedures, for example, translation, back translation, and pilot study. This procedure has been finished. The second step will be to compare the quality of diabetes care between diabetes specialists and primary-care physicians as non-specialists. The last step will be to perform a cluster randomized controlled trial to evaluate the efficiency of the chronic care model.

The effect on patient satisfaction of communication styles in medical interviews At present, the patient-physician relationship is of great interest in Japan. However, few studies have been reported. This study used the Roter Interactional Analysis System to evaluate the effect on patient satisfaction of communication styles in the medical inter-

Publications

view.

Sato M, Yamadera W, Matsushima M, Itoh H, Nakayama K. Clinical efficacy of individual cognitive behavior therapy for psychophysiological insomnia in 20 outpatients. *Psychiatry Clin Neurosci* 2010; **64:** 187-95.

Nakayama M, Sato T, Sato H, Yamaguchi Y, Obara K, Kurihara I, Sato K, Hotta O, Seino J, Miyata M, Takeuchi K, Nakayama K, Matsushima M, Otaka T, Kinoshita, Y, Taguma Y, Ito S. Different clinical outcomes for cardiovascular events and mortality in chronic kidney disease according to underlying renal disease: the Gonryo study. Clin Exp Nephrol 2010; 14: 333-9.

Ogawa S, Nakayama K, Nakayama M, Mori T, Matsushima M, Okamura M, Senda M, Nako K, Miyata T, Ito S. Methylglyoxal is a predictor in type 2 diabetic patients of intima-media thickening and elevation of blood pressure. *Hypertension* 2010; **56**: 471–6.

Inoue T, Kawai M, Nakane T, Nojiri A, Minai K, Komukai K, Ogawa T, Hongo K, Matsushima M, Yoshimura M. Influence of low-grade inflammation on plasma B-type natriuretic peptide levels. Intern Med 2010; **49:** 2659-68.

Laboratory Animal Facilities

Kiyoshi Ohkawa, Professor and Director

Tatsuya Furuichi, 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 2010, 173 researchers used the LAF. We undertake breeding of experimental animals and technically guide researchers in animal experimentation. In addition, we performed the following studies to develop basic medical sciences, including laboratory animal science.

Research Activities

Identification of a novel Col2a1 mutant mouse

In the RIKEN N-ethyl-N-nitrosourea (ENU) mutagenesis project, we identified a novel Col2a1 mutant mouse (termed M856). The M856 mouse has a missense mutation, c.4406A>C (p.Asp1469Ala), in the C-propeptide coding region of Col2a1 and in the position corresponding to human COL2A1 mutation (p.Asp1469His) responsible for platyspondylic dysplasia, Torrance type (PLSD-T). The M856 homozygote exhibits lethal skeletal dysplasias, including extremely short limbs, severe spondylar dysplasia, and severe hypoplasia of the pelvis. As expected, these defects were similar to those in patients with PLSD-T. The M856 mutant should serve as a good model to study PLSD-T.

Generation of Cant1 knockout mice

Desbuquois dysplasia is a severe skeletal dysplasia inherited in an autosomal recessive manner, characterized clinically by severe growth retardation, joint laxity, flat midface, and characteristic hand abnormalities. Recently, mutations in the gene that encodes for calcium-activated nucleotidase 1 (CANT1) have been identified in a subset of patients with Desbuquois dysplasia. CANT1 is an extracellular protein that functions as a nucle-otide triphosphatase and diphosphatase. It preferentially hydrolyzes uridine diphosphate followed by guanidine diphosphate, uridine triphosphate, and ADP. To investigate the in vivo function of CANT1, we started to generate Cant1 knockout mice. By using the inverse polymerase chain reaction and rapid amplification of 5'-complementary DNA ends, we isolated Cant1 knockout embryonic stem cells from the Gene-Trap ES Library.

Establishment and characterization of strains originated from Japanese wild mice and Phodopus hamster

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

Japanese wild mice are classified as *M. m. molossinus* and originated from a natural intersubspecific hybrid between *Mus musculus castaneus* inhabiting southwest Asia and *Mus* *musculus musculus* distributed in north Asia. The *molossinus* subspecies is an excellent source for improving laboratory mice, because it was suspected to be greatly different in gene constitution from common laboratory mice derived from the *Mus musculus domesticus* subspecies. We have established several new inbred strains based on *molossinus* mice captured in Osaka prefecture. These strains are being maintained in our laboratory, and new consomic strains based on these strains are being developed.

In collaboration with the Department of Molecular Biology, we developed 2 new mouse strains using our original *molossinus* inbred strain named MSKR. One is the congenic strain having knockout allele of *Oaz1* derived from the B6.129-*Oaz1^{im}* to the MSKR background, and the other is a consomic strain that has chromosome 10 derived from the above-mentioned strain to the MSKR background. We have confirmed that these newly established strains are useful for researching genetic modifications in *Oaz1* knockout mice.

The *Phodopus* hamster is a small rodent that differs taxonomically from the Syrian hamster, which is the major laboratory hamster. We recently determined that this hamster is a good candidate for a new laboratory animal and have established an inbred strain. Furthermore, we continue to establish new inbred strains and congenic strains and to develop models of human diseases to research their 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 separate lines in each laboratory. The NC/Nga inbred strain maintained in our laboratory has an extremely severe dermatitis diathesis. In collaboration with the Department of Tropical Medicine, we are using the NC/Nga mice to research new drugs for treating atopic dermatitis.

Publications

Furuichi T, Dai J, Cho TJ, Sakazume S, Ikema M, Matsui Y, Baynam G, Nagai T, Miyake N, Matsumoto N, Ohashi H, Unger S, Superti-Furga A, Kim OH, Nishimura G, Ikegawa S. CANT1 mutation is also responsible for Desbuquois dysplasia, type 2 and Kim variant. J Med Genet 2011; **48**: 32-7.

Okada I, Hamanoue H, Terada K, Tohma T, Megarbane A, Chouery E, Abou-Ghoch J, Jalkh N, Cogulu O, Ozkinay F, Horie K, Takeda J, Furuichi T, Ikegawa S, Nishiyama K, Miyatake S, Nishimura A, Mizuguchi T, Niikawa N, Hirahara F, Kaname T, Yoshiura K, Tsurusaki Y, Doi H, Miyake N, Furukawa T, Matsumoto N, Saitsu H. SMOC 1 is essential for ocular and limb development in humans and mice. Am J Hum Genet 2011; 88: 30-41. Wada A, Ohkawa K, Tsudzuki M (Hiroshima Univ). Establishment and genetic characterization of the new inbred strain originated from Phodopus campbelli. Exp Anim 2010; 59: 318. Wada A, Ohkawa K, Tsudzuki M (Hiroshima Univ). Sequencing of the attractin gene in the black coat color mutant Phodopus campbelli. Gene Genet Sys 2010; 85: 411.

Watanabe Y, Takeuchi K, Higa Onaga S, Sato M, Tsujita M, Abe M, Natsume R, Li M, Furuichi T, Saeki M, Izumikawa T, Hasegawa A, Yokoyama M, Ikegawa S, Sakimura K, Amizuka N, Kitagawa H, Igarashi M. Chondroitin sulfate N-acetylgalactosaminyltransferase-1 is required for normal cartilage development. Biochem J 2010; **432:** 47–55.

Radioisotope Research Facility

Kunihiko Fukuda, Professor and Director

Yukio Yoshizawa, Assistant Professor

General Summary

The Radioisotope Research Facility was established to support medical and biological research with radioisotopes. 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 2010, 28 researchers from 9 departments and 12 students of 2 curricula used the laboratory in this facility. Major nuclides used for experiments were ³²P, ⁵¹Cr, ¹²⁵I, ³⁵S, and ³H.

Research Activities

The active site of exfoliative toxin A of Staphylococcus aureus

Exfoliative toxin A (ETA) of *Staphylococcus aureus* is the causative agent of staphylococcal scalded-skin syndrome (SSSS) and disrupts desmosomes to half desmosomes in neonatal mouse epidermal tissue injected with ET and in patients with SSSS. We have previously reported that the nitration of tyrosine (Tyr) residues causes ETA to lose all toxicity. To determine the biologically active site of ETA, we prepared mutant ETA using oligonucleotide primers designed to introduce mutations with the polymerase chain reaction. Substitution of serine (Ser)-195, which is believed to be the active center of the serine protease, and other 16 Ser residues had no effect on ETA activity or immunoreactivity with anti-ETA rabbit serum. On the other hand, substitution of Tyr-17-18 and Tyr-225-232 destroyed both activity and immunoreactivity.

The promoter region of the *eta* gene consists of -35 TTGTTT, -10 TATAAT, and S-D sequence GGATGA, and an inverted sequence is present between the -10 and the S-D sequences. There is an open reading frame of 1,382 bp adjacent to the *eta* promoter. Without this gene, ETA production decreases markedly. A gel mobility shift assay was performed with the gene product ETAexp and a DNA fragment of 381 bp including the *eta* promoter sequence. Results indicate that ETAexp bound to the inverted sequence and activated the transcription of the *eta* gene.

Development of techniques for determining radioactivity

Radon gas emanates from the ground directly or is brought by groundwater and mineral water to the surface of the earth and released into the atmosphere. Radon is the second leading cause after smoking of lung cancer in the general population. A reference level of 300 Bq/m³ was proposed to minimize the health hazard due to indoor radon exposure (International Commission on Radiological Protection, 2009). Liquid scintillation counting has been a standard method for measuring radon in air and water. The popularity of this method is due to the high solubility of radon in scintillation solvents, such as

toluene and xylene. These solvents, however, could not satisfy safety requirements because of their volatile nature and low flash points. We have used silicone oil as scintillation solvent to overcome these undesirable properties. The open-vial method was applied to measure radon in air by Horiuchi and Murakami in 1983. The toluene scintillator used in their study evaporated during the exposure, and correction of the toluene quantity was required. This correction was unnecessary when the silicone oil scintillator was applied for the open-vial method. No evaporation of silicone oil was observed during 48 hours' exposure. The open-vial method using the silicone oil scintillator was sensitive enough to detect radon at a level of 160 Bq/m³.

Study of resistance mechanisms in radiation-resistant organisms

Tardigrades show remarkable adaptability under extreme environmental conditions, such as high radiation, high temperature, and high pressure. Activated sludge was obtained from the Ariake Water Reclamation Center of the Tokyo Metropolitan Government and used to collect tardigrades. Tardigrades isolated from activated sludge were identified with 18S-ribsomal DNA as *Isohypsibius*. *Isohypsibius* from activated sludge assume a barrel-like form, but not the complete 'tun' (that means barrel shaped tardigrade) under drying conditions. We examined the effects of radiation on the tardigrade with ⁶⁰Co irradiation at the Takasaki Advanced Radiation Research Institute. *Isohypsibius* tardigrades exposed to radiation showed a moderately higher survival rate than did those not irradiated.

Core Research Facilities

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

General Summary

The Research Center for Medical Sciences was reorganized as the Core Research Facilities, which consist of the Divisions of Fine Morphology, Biochemistry, and Advanced-Research Laboratory. The mission of the facilities is the facilitation of research in the university. Two systems are constituted for use of the facilities.

Annual Registration System

This system is intended for researchers in the university to supply research space, benches, and other equipment to perform experiments. Once registered, researchers can freely use the various devices, such as fluorescent microscopes, optical microscopes, and equipment to prepare specimens for histological examinations, high-performance liquid chromatography is used, and nucleic acid amplification system (polymerase chain reaction). Because inspections and maintenance are regularly performed by the staff, equipment is in excellent condition and available at any time. In addition to providing space, devices, and equipment, this system can provide technical advice and guidance on specific fine-morphological or biochemical approaches to registrant's experiments.

Research Service-Providing System

Advances in research technologies and hardware enables us to perform more detailed and accurate observations of specimens in medical sciences. However, various technologically highly-advanced devices require specialized knowledge. These advances sometimes cost researchers both time and money. Also, all researchers are not necessarily familiar with all the equipment available for medical experimentation. For researchers who cannot perform experiments because of limitations of time and resources, our staff can prepare specimens for scanning electron microscopy and transmission electron microscopy, record images, or perform high-performance liquid chromatography and mass spectrometry for analysis. By the use of this system, researchers can proceed efficiently. The service fee is minimal because services are limited to the university.

Research Activities

Conjugation of fluorescent nanoparticles to monoclonal antibodies

Fluorescent nanoparticles have been used as tracers for the visualization, qualification, and quantification of biological molecules. We conjugated particles to the monoclonal antibody JT95 to investigate thyroid papillary carcinoma. The antibody recognizes antigens of gylcosylated fibronectin produced by thyroid carcinoma and can be used as a biomarker for detecting the tumor. The antibody-nanoparticle conjugate is also useful for

evaluating tumor progression or recurrence after surgery. Several methods for conjugation were tested, and we optimized the conditions.

Functional analysis of tight junctions

Tight junctions (TJs) among adjacent epithelial cells control paracellular permeability of solutes. Epidermal TJs are believed to restrict molecular movement to assist the stratum corneum as a secondary barrier in the skin. However, the role of TJs in molecular distribution in the epidermis has not been thoroughly studied. Calcium ions (Ca^{2+}) , which induce keratinocyte differentiation, are distributed in a vertical gradient peaking in the stratum granulosum. In this study, we applied sodium caprate (C10), which elicits dilation of TJs on human reconstructed epidermis, and investigated Ca²⁺ distribution in the epidermis. The localization of Ca^{2+} in the epidermis was observed with ion-capture cytochemistry and electron energy-loss spectroscopy with a transmission electron microscope. After treatment with C10, the epidermal Ca^{2+} localization was altered compared with that in untreated epidermis. Precipitates containing Ca^{2+} appeared in the intracellular and extracellular spaces of the stratum corneum, and large clusters of these precipitates were occasionally observed in the stratum corneum and the stratum granulosum. 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 epider-We found that the TJ strands were fragmented and that the Ca²⁺ permeability had mis. These findings suggest that epidermal TJs maintain Ca²⁺ under the stratum increased. corneum and regulate epidermal differentiation.

Adhesion and structural properties of protein nanomaterials containing hydrophobic and charged amino acids

Protein polymers are being used or considered for biologically based adhesives and coating materials. Most adhesives derived from macroprotein molecules work through receptors or cross-links. Clarifying the adhesion mechanism of protein polymers would lead to a better understanding of adhesives and the discovery of new practical properties of protein polymers at both the nanoscopic and macroscopic levels. The objective of this research project was to study the adhesion properties of protein polymers at the nanoscopic level. Seven protein nanomaterial samples with different degrees of adhesive strength were designed and synthesized using solid phase chemistry. All protein nanomaterials contain a common hydrophobic core flanked by charged amino acid sequences. The adhesion properties of the protein nanomaterials were investigated at different pH values and curing temperatures. The protein nanomaterials self-aggregate and interact with a wood surface. The protein nanomaterial KKK-FLIVIGSII-KKK identified in this study had high adhesive strength toward wood. It had the highest shear strength at pH 12, with an amino acid sequence that was extremely hydrophobic and uncharged. This protein nanomaterial was subjected to structural analyses using circular dichroism, laser-Fourier transform infrared spectroscopy, and matrix-assisted laser desorption ionized mass spectrometry. At pH 12 this peptide adopted a pH-induced beta-sheet-like conformation. Adhesive strength reflects contributions of both hydrogen bonding and van der Waals interactions. Our findings suggest that ionic and covalent bonds are not significant factors in adhesion.

Publications

Shirasu M, Fujioka K, Kakishima S, Nagai S, Tomizawa Y, Tsukaya H, Murata J, Manome Y, Touhara K. Chemical identity of a rotting animallike odor emitted from the inflorescence of the titan arum. *Biosci Biotechnol Biochem* 2010; **74**: 2550-4.

Inaba N, Ishizawa S, Kimura M, Fujioka K, Watanabe M, Shibasaki T, Manome Y. Effect of inhibition of the ROCK isoform on RT2 malignant glioma cells. *Anticancer Res* 2010; **30**: 3509-14.

Hoshino A, limura T, Ueha S, Hanada S, Maruoka Y, Mayahara M, Suzuki K, Imai T, Ito M, Manome Y, Yasuhara M, Kirino T, Yamaguchi A, Matsushima K, Yamamoto K. Deficiency of chemokine receptor CCR1 causes defective bone remodeling due to impaired osteoclasts and osteoblasts. J Biol Chem 2010; **285**: 28826-37.

Raleigh DR¹, Marchiando AM¹, Zhang Y¹, Shen L¹, Sasaki H, Wang Y¹, Long M¹, Turner JR¹ ('Chicago Univ). Tight junction-associated MARVEL proteins marveld3, tricelulin, and occludin have distinct but overlapping functions. *Mol Biol* Cell 2010; **21**: 1200–13.

Muto S¹, Hata M¹, Taniguchi J¹, Tsuruoka S¹, Moriwaki K¹, Saitou M², Furuse K¹, Sasaki H, Fujimura A¹, Imai M¹, Kusano E¹, Tsukita S², Furuse M¹ ('Kobe Univ, ²Kyoto Univ). Claudin-2-deficient mice are defective in the leaky and cation-selective paracellular permeability properties of renal proximal tubules. *Proc Natl Acad Sci* USA 2010; **107:** 8011–6.

Shiozuka M¹, Wagatsuma A¹, Kawamoto T¹,

Sasaki H, Shimada K¹, Takahashi Y¹, Nonomura Y¹, Matsuda R¹ (¹Univ Tokyo). Transdermal delivery of a readthrough-inducing drug: a new approach of gentamicin administration for the treatment of nonsense mutation-mediated disorders. *J Biochem* 2010; **147**: 463-70.

Wu S¹, Liu J¹, Reedy MC², Tregear RT³, Winkler H¹, Franzini-Armstrong C⁴, Sasaki H, Lucaveche C¹, Goldman YE⁴, Reedy MK², Taylor KA¹ ([†]Florida Univ, ²Duke Univ, ³MRC, ⁴Univ Pennsylvania). Electron tomography of cryofixed, isometrically contracting insect flight muscle reveals novel actin-myosin interactions. *PLoS One* 2010; **5**: e12643.

Iwase T, Shinji H, Tajima A, Sato F, Tamura T, Iwamoto T, Yoneda M (Univ Tokyo), Mizunoe Y. Isolation and identification of ATP-secreting bacteria from mice and humans. *J Clin Microbiol* 2010: **48:** 1949-51.

Tomich J^I, Iwamoto T, Shen X^I, Susan San X^I ('Kansas State Univ). PH dependent adhesive peptides. United States patent US 7745570. 2010 June 29.

Herrera AI¹, AI-Rawi A¹, Cook GA², Gao J¹, Iwamoto T, Prakash O¹, Tomich JM¹, Chen J¹ ('Kansas State Univ, ²Univ San Diego). Structural characterization of two pore-forming peptide: Consequences of introducing a C-terminal tryptophan. Proteins 2010; **78**: 2238-50.

Ayella A¹, Lim S¹, Jiang Y¹, Iwamoto T, Lin D¹, Tomich J¹, Wang W¹ (¹Kansas State Univ). Cytostatic inhibition of cancer cell growth by lignan secoisolariciresinol diglucoside. Nutr Res 2010; **30**: 762-9.

Department of Genetic Diseases and Genome Science

Yoshikatsu Eto, Professor and Director Takashi Higuchi, Postdoctoral Fellow Torayuki Okuyama, Visiting Professor Collaborating Researchers Hiroyuki Ida, Professor Toya Ohashi, Professor Hiroshi Kobayashi, Assistant Professor Masayuki Kobayashi, Assistant Professor

General Summary

The main research topics in the Department of Genetics and Genome Science are the basic pathogenesis of genetic diseases, particularly, lysosomal storage diseases (LSDs), and the development of therapies for LSDs. Among them, the pathogenesis of central nervous system (CNS) involvement in LSDs is the most important research subject. To understand the pathophysiology of CNS events, we generated induced pluripotent stem (iPS) cells from mucopolysaccharidosis (MPS) VII mice and differentiated them into neuronal cells. We also generated iPS cells from model mice of Pompe disease and differentiated them into skeletal muscle cells. We can produce disease models of various LSDs using iPS technology. Furthermore, we treated CNS involvement of LSDs by means of intrathecal injection of enzymes into MPS II mice. These findings indicate that intrathecal treatment is feasible to treat the CNS in various LSDs.

Research Activities

1. Development of treatment procedures for LSDs

To establish novel treatment procedures for CNS involvement of LSDs is our most important project. One procedure is intrathecal or intraparenchymal injection of enzymes into MPS II mice. We found that intrathecal injection produced significant elevations of enzyme activities in various regions of the brain and in other organs, such as the liver, spleen, kidney, and heart. Furthermore, histological correction in the brain was observed.

2. The iPS cells from various LSDs might provide insights into the pathophysiology of LSDs and might be used to treat them. We successfully generated iPS cells from Pompe mice using tail-tip fibroblasts and mouse embryonic fibroblasts. With 3 factors—Klf4, Sox2, and Oc2/4t—we caused the iPS cells to differentiate into skeletal muscle cells. Pompe skeletal muscle cells showed massive accumulation of glycogen in lyso-somes surrounded by a single membrane unit.

3. The screening for LSDs with dried blood spots is an important technology for the early diagnosis and treatment of patients with various LSDs. We used the fluorometric assay method to establish the dried blood spot method for the early diagnosis of Pompe disease, Fabry disease, Morquio syndrome, and MPS VI.

Publications

Ohashi T, lizuka S, Shimada Y, Eto Y, Ida H, Hachimura S, Kobayashi H. Oral administration of recombinant human acid a-glucosidase reduces specific antibody formation against enzyme in mouse. *Mol Genet Metab* 2011; **103**: 98-100. Epub 2011 Jan 27.

lida T, Shiba H, Misawa T, Ohashi T, Eto Y. Immunogene therapy against colon cancer metastasis using an adenovirus vector expressing CD40 ligand. *Sugery* 2010; **148**: 925-35.

Kobayashi H, Takahashi-Fujigasaki J, Fukuda T, Sakurai K, Shimada Y, Nomura K, Ariga M, Ohashi T, Eto Y, Otomo T, Sakai N, Ida H. Pathology of the first autopsy case diagnosed as mucolipidosis typelll α/β suggesting autophagic function. *Mol Genet Metab* 2011; **102:** 170-5.

Meng XL, Shen JS, Kawagoe S, Ohashi T, Brady R, Eto Y. Induced pluripotent stem cells derived from mouse models of lysosomal storage disorders. *Proc Natl Acad Sci U S A* 2010; **107:** 7886-91.

Kyosen SO, lizuka S, Kobayashi H, Kimura T, Fukuda T, Shen JS, Shimada Y, Ida H, Eto Y, Ohashi T. Neonatal gene transfer using lentiviral vector for murine Pompe disease: long term expression and glycogen reduction. *Gene Ther* 2010; **17:** 521-30.

Okuyama T, Tanaka A, Suzuki Y, Ida H, Tanaka T, Cox GF, Eto Y, Orii T. Japan Elaprase Treatment (JET) study: idursulfase enzyme replacement therapy in adult patients with attenuated Hunter syndrome (Mucopolysaccharidosis II, MPS II). Mol Genet Metab 2010; 99: 18-25.

Eto Y. Single gene disorder: recent advances of research(in Japanese). *Nippon Rinsho* 2010; **68 Suppl 8:** 117-28.

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

Interventional effect of resistance exercise on protein expression levels in adult and old rat skeletal muscles atrophied by hindlimb unloading

We examined expression levels of the key proteins involved in cellular adaptation in adult and old rat skeletal muscles atrophied by hindlimb unloading and the effect of resistance exercise on muscle atrophy. Female F344 rats (5 and 24 months old) were divided into 3 groups: a control group, a hindlimb-unloading group (HU group), and a hindlimb-unloading with resistance exercise group (HU+RE group). Rats in the HU group and the HU+RE group had their hindlimbs suspended for 3 weeks. RE was performed daily as three 10-minute exercises, every 4 hours (total, 30 minutes/day) during the hindlimb-unloading The lateral gastrocnemius muscles were dissected out, separated into white and period. red regions, and then homogenized. The protein expression levels were determined with Western blotting. In white regions of the lateral gastrocnemius, group differences in Akt activity (phospho-Akt/pan Akt ratio) and myostatin expression were not detected at either 5 or 25 months. In red regions of the lateral gastrocnemius, Akt activity was lower in the HU group than in the control group, and myostatin expression was lower in the HU+RE group than in the HU group at 24 weeks but not at 5 weeks. Expression of heat shock protein 72 in both red and white muscle regions were in the order of HU group<control group<HU+RE group. Expression of peroxisome proliferative activated receptor- γ coactivator 1 in red regions of the lateral gastrocnemius were in the order of HU group< HU+RE group<control group. The present results suggest that these proteins play physiological roles in cellular adaptation induced by hindlimb unloading and resistance exercise.

Effects of exercise training and protein malnutrition on myostatin expression in rat skeletal muscles

Myostatin, a member of the transforming growth factor-beta superfamily, plays an important role in regulating skeletal muscle mass. Here we examined the role of myostatin in the change in rat skeletal muscle mass induced by exercise training and protein malnutrition. Female F344 rats (6 weeks old) were divided into a normal diet group or a lowprotein diet group, which were then subdivided into an untrained control group or a trained group. The rats of the trained group ran on a motor-driven treadmill (35 m/minute) 5 days per week for 8 weeks. The soleus, plantaris, and medial gastrocnemius muscles were extracted and weighed. The fiber size of each muscle fiber type identified with staining for myofibrillar ATPase was measured. Myostatin expression was determined with Western blotting. Protein malnutrition inhibited increases in muscle mass of all muscles and inhibited increases in the fiber size of all fiber types with growth. Running training resulted in massive increases of the soleus and medial gastrocnemius muscles, but not of the plantaris muscle. Fiber size in almost all fiber types increased with running training. Myostatin expression in all muscles decreased with running training but not with protein malnutrition. The present results suggest that myostatin regulates skeletal muscle mass in response to mechanical stress but not in response to nutritional condition.

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 (1) gravitational physiology and aerospace medicine, and (2) physioepidemiological studies of health.

Research Activities

Gravitational physiology and aerospace medicine

1. Technique of electrocardiography in medaka

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

The medaka was placed in a damp sponge, and bipolar-lead (I, II, III, and NASA) ECGs were recorded under unanesthetized conditions with needle electrodes inserted through the skin. Wave-form analysis was performed with PowerLab data acquisition software (ADInstruments, Sydney, Australia).

In this study, we recorded clear ECG data with the NASA lead but not with leads I, II, or III. Because the data quality might depend on the needle position, we should develop a technique for precise needle insertion.

2. Research on visual stimuli and posture control

Information for maintaining body direction and movement of the body center to maintain posture are determined by visual input factors, equilibrium vestibular input factors, and somatosensory factors from the whole body (including muscle, tendon, joints, and skin). Visual information becomes the main factor in outer space because vestibular and somatosensory inputs are reduced because of low or absent gravity. The objective of this research is to analyze changes in posture induced by visual stimuli.

3. Outreach activities for aerospace medicine

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

Results of physioepidemiological studies

Many previous studies of wellness medicine and occupational health have been performed with epidemiological methods. However, in this study physiological data (e.g., ECG) were analyzed with epidemiological methods. Mental stress and human health can be evaluated objectively using both physiological and epidemiological methods.

1. Comparison of ECGs from parents and children

The QTc interval determined from ECGs showed a significant relationship between parents and children. The QTc interval might be affected by hereditary factors.

2. Mental stress in physicians performing endoscopic procedures

The mental stress experienced by endoscopists performing endoscopic procedures was directly measured with Holter ECG monitors. Performing an endoscopic procedure causes endoscopists to experience mental stress. This stress may be severe until the endoscopist has acquired significant experience.

Publications

Toi T¹, Nomura Y¹, Sudoh M, Ikeda A¹, Masuda T¹, likuni H¹, Asakawa N¹, Ikui A², Shigihara S¹, Ikeda M¹ (¹Nihon Univ Sch Med, ²Hakuraku *Clin).* A case of vertigo and hearing loss induced by aero-alternobaric barotraumas (in Japanese). *Uchu Koku Kankyo Igaku* 2010; **47:** 25-31.

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. Since 2010, we have also focused on issues related to sports injuries in elderly athletes.

Research Activities

Three-dimensional motion analysis of the pelvis during soccer kicks

We performed 3-dimensional motion analysis of the pelvis during soccer kicks using a 200-Hz motion analysis system (Vicon Motion Systems, Oxford, UK) to evaluate the mechanical stress around the pelvis. Mechanical stress to the hip adductor muscles during the infront kick was greater than that during the instep kick. In addition, during the infront kick, mechanical stresses to those muscles were greater if the kickers did not use their upper extremities. We speculate that the infront kick without the use of the upper extremities might contribute to injuries of the hip adductors.

Three-dimensional gait analysis in patients with bilateral knee osteoarthritis before and after unilateral total knee arthroplasty

The purpose of this study was to compare the data of 3-dimensional gait analysis with a motion analysis system (Vicon Motion Systems) in 26 patients with bilateral knee osteoarthritis who had undergone unilateral total knee arthroplasty. Analyzed variables were: 1) step length, 2) walking speed, 3) percentage of the gait in the single-limb support (SLS) phase, 4) ground force during the SLS phase, 5) step width, and 6) range of motion of the hip, knee, and ankle joints. Step length, walking speed, percentage of the gait in the SLS phase, ground force during the SLS phase, and range of motion improved significantly in patients with a Japanese Orthopaedic Association score of 60 points or more for the side not operated on. On the other hand, patients with a score of less than 60 points showed no improvement in the aforementioned variables. We conclude that various gait variables improve after total knee arthroplasty, although patients with severe osteoarthritis (on the side not operated on) showed no improvement in their walking ability.

Comparison of core muscle strength between baseball players and soccer players

We compared core muscle strength between baseball players and soccer players. The subjects were 20 players of each sport, with an average age of 16 years. The lengths of time the subjects could maintain the lower limb in the "bench" and "sideways bench"
positions were measured. The times for both the bench and sideways bench positions were significantly longer for soccer players than for baseball players. We conclude that these differences were caused by the characteristics of these sports.

Treatment of elder athletes in our clinic

We evaluated 597 patients who were older than 40 years and had visited our clinic for the past 3 years. The majority of the patients suffered from osteoarthritis of the lumbar spine or knee joints. They were treated with injections of hyaluronic acid or medication or both. In addition, athletic rehabilitation, such as exercises to increase range of motion and balanced training, were also applied and were useful in this patient group. Rehabilitation should be continued even after the return to their sports activities.

Fracture dislocation of the Lisfranc joint in a young soccer player

We reported a rare case of fracture dislocation of the Lisfranc joint with the fracture of the first cuneiform. The mechanism of this injury was investigated.

A report of a cycle racer associated with myasthenia gravis who resumed professional racing We reported on a cycle racer who had myasthenia gravis, was treated with muscle training during hospitalization, and finally returned to professional racing.

Publications

Funasaki H, Yoshida M, Kan I, Kato S, Moro-hashi M, Kasama K, Marumo K. Arthroscopic Bankart repair for recurrent shoulder dislocation in patients over 40 years of age (in Japanese). *Katakansetsu* 2010; **34:** 355–8.

Yoshida M, Funasaki Y, Kan I, Kato S, Kasama K, Marumo K. Gene expression analysis for thick subacromial bursae accompanied with rotator cuff tear (in Japanese). JOSKAS 2011; 36:

22-3.

Reviews and Books

Funasaki H. Case presentation —my opinion— (in Japanese). In: Tamai K, editor. Proximal humeral fracture. Tokyo: Kanehara Shuppan; 2010. p. 166.

Health-Care Center

Mikio Zeniya, Professor and Director Takashi Wada, Professor Hiroki Takahashi, Assistant Professor Hiroko Nogi, Assistant Professor Yoich Sakamoto, Professor Takekazu Onda, Professor Kazumi Kawase, Assistant Professor

General Summary

Shimbashi Medical Checkup Office

1. Preventive Medicine

We focused on metabolic syndrome, smoking, and lifestyle changes to prevent lifestylerelated disease. In April 2005, the Japanese Society of Internal Medicine and 8 other Japanese medical societies defined Japan-specific diagnostic criteria for metabolic syndrome. The criterion of waist circumference that indicates accumulating abdominal visceral fat has been discussed. We validated the evidence with the objects of occurrence of dyslipidemia, high serum glucose levels, and high blood pressure.

Research Activities

Shimbashi Medical Checkup Office

1. Metabolic syndrome

We performed a retrospective cohort study to investigate the relation of the cut-off value of waist circumference to the prevalence of dyslipidemia. The subjects, who had an annual medical checkup at our center from 2000 through 2008 and had no dyslipidemia at their initial examination, were extracted. Dyslipidemia was defined as a triglyceride (TG) level≥150 mg/dL, a high-density lipoprotein cholesterol (HDL-C) level<40 mg/dL, or treatment. The subjects were divided into 2 groups, a nononset group that showed no dyslipidemia and an onset group in which dyslipidemia developed in a time span of three years. We calculated the cut-off point between the nononset group and the onset group from the receiver operating characteristic curve, concerning abdominal circumference, body mass index (BMI), and the waist/height ratio. The cut-off values for the TG level were as follows: an abdominal circumference of 84 cm in men and 78 cm in women, a BMI of 23.5 kg/m² in men and 21 kg/m² in women, and a waist/height ratio of 0.5 in men and 0.49 in women. The cut-off values for HDL-C were as follows: an abdominal circumference of 85 cm in men and 79 cm in women, a BMI of 24.0 kg/m² in men and 21.5 kg/ m^2 in women, and a waist/height ratio of 0.5 in men and 0.5 in women. For both TG and HDL-C, the area under the receiver operating characteristic curve of the abdominal circumference was the highest among the 3 indexes.

Publications

Wada T. Lung age in smokers is 10 years older, and 5 years older in even quitting smoking (in Jap-

anese). Igaku no Ayumi 2010; **233:** 1107-8. *Fukumoto T, Joki M, Inaji J, Nakazaki K,* Hashimoto H, Mashima K, Toyohara K, Zeniya M, Wada T, Sugimori H. Reference interval of lung age in non-smoker (in Japanese). *Ningen Dock* 2010; **25:** 676-80. *Nakazaki K, Wada T.* Behavior therapy—per-

Nakazaki K, Wada T. Behavior therapy—personal type diet (in Japanese). *Sogo Rinsho* 2010; **59:** 1974-7.

Reviews and Books

Wada T. Lifestyle and strategy for the prevention of obesity (in Japanese). In: Ikeda Y, editor. Medicine in obesity. Tokyo: Nippon Hyoronsha; 2011. p. 77-85.

Premedical Course

Biology

Osamu Terasaka, Professor

Rie Hiratsuka, Assistant Professor

General Summary

The main research subject of our laboratory is the reproductive system of seed plants. Our research is now focused on the relation between pollen tube growth and the programmed cell death of pollen tube conducting tissue.

Research Activities

Role of the pectinase (Cry j 1, Cry j 2) in pollen tube growth of Cryptomeria japonica Cry j 1 and Cry j 2 are the major allergic proteins of *Cryptomeria japonica* pollen. These proteins are presumed to play important roles in pollen germination and tube growth because they have pectinase activity. Their distribution patterns shown *in vitro* suggest that Cry j 1 and Cry j 2 are not involved in pollen germination and tube growth. However, their pectinase activities suggest that Cry j 1 and Cry j 2 may facilitate the growth of the gymnospermous pollen tube in the nucellus through the degradation of nucellar pectins. In the present study, we examined the *in vivo* localization of Cry j 1, Cry j 2, and pectins in the pollen tube and the nucellus of *C. japonica* to clarify the role of these pectinases.

Cry j 2 was detected in the cytoplasm of the pollen tube, tube wall, nucellar cells that came in contact with a pollen tube, and the middle lamella. In contrast, Cry j 1 was not observed throughout the pollen. De-esterified pectins were localized in nucellar cell walls, and methyl-esterified pectins were localized in nucellar cell walls and the middle lamella. These findings indicate that Cry j 2 is synthesized in the pollen tube and is secreted to the nucellus via the tube wall and degrades pectins of the nucellar cell wall and the middle lamella to facilitate pollen tube growth. The absence of Cry j 2 in the pollen tube wall in vitro also indicates that interaction between the pollen tube and the nucellus is necessary to induce the secretion of Cry j 2.

Publications

Hiratsuka R, Terasaka O. Pollen tube reuses intracellular components of nucellar cells undergoing programmed cell death in *Pinus densiflora*. *Protoplasma* 2011; **248:** 339-51. Epub 2010 Jul 10.

Physics

Koichi Satoh, Professor

Katsumi Kasono, Assistant Professor

General Summary

1. Dipalmitoylphoshatidycholine (DPPC) membranes have been studied from several points of view, including formation, liquid crystals, phase transitions, interaction with ions, and optical characteristics.

2. Phase transitions, critical phenomena, interacting many-body systems, computer simulation.

Research Activities

Ripple phase of the DPPC membrane

The ripple phase of the DPPC membrane is assumed to be caused by the presence of about 20% fluid lipids in the gel phase. This assumption consistently explains 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 made cluster update and Metroplice single-spin update simulations to the study of systems with the first-order phase transition. We calculated surface tension of a square lattice.

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

Theoretical analysis of molecular interactions of Cu(II) bis(diarylpropanedionedioate) complexes with benzene ligands

Arene-polyfluoroarene interaction was found between the Cu(II) bis[3-oxo-1,3-bis(pentafluorophenyl)prop-1-en-1-olate] complex and benzene as the guest molecule.

This weak interaction occurs only when 2 fluorine substituents exist at the o- and o'-positions of the ligand aryl groups. Density functional theory calculation predicted larger torsional angles of aryl rings against the central square-planar Cu(II) complex in o,o'-difluorinated phenyl diketonate complexes than in the parent phenyl diketonate complexes. This distortion makes a binding pocket for a guest benzene molecule to form stable host-guest complexes.

Synthesis of N-protected peptide acids using amino acid-alkaline earth metal salts

The protection of a carboxyl group by a metal ion saves the time for the incorporation and removal of the protecting group and prevents side reactions caused by the use of esters. The syntheses of *N*-protected peptide acids in organic solvents using alkaline earth metal-carboxylate salts of amino acids were investigated. We found that the amino acid-Ca carboxylate salts is are the most effective among 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.

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, judicial system, and local government. I participate in the "Unequal Society and Safety-Net" working party and the "Structural Change of Publics" working party of the Legal Committee of the Science Council of Japan.

Reviews and Books

Ozawa R. Argument over article 9 reformation of Japanese constitution, past and present (in Japanese). In: Japan Scientists' Association, editor. Kenpou to Genjitsu Seiji. Tokyo: Honnoizumisha; 2010. p. 83-92.

Ozawa R. Political right and democracy of today (in Japanese). In: Japan Scientists' Association, editor. Kenpou to Genjitsu Seiji. Tokyo: Honnoizumisha; 2010. p. 214-33. **Ozawa R.** Japan-U.S. security system and financial control (in Japanese). In: The Law Section of the Association of Democratic Scientists, editor. Anpo kaitei 50 nen. Tokyo: Nippon Hyoronsha; 2010. p. 111-6.

Ozawa R, Maruyama S. Minshutou seikenka no nichibei-anpo (in Japanese). Tokyo: Kadensha; 2011.

Human Science

Takao Fukuyama, Professor

General Summary

The study of Western philosophy and ethics

Research Activities

The feeling of trust in the world

I study several aspects of the human relationships of support and care. Through this support of others, people acquire the feeling of trust in their world. However, when people have failed to earn this support, they cannot lead an ordinary life. For example, abused babies, bullied school children, battered women, and patients with life-threatening illnesses can be cured after learning to trust others.

Philosophically, these situations require a new theory of relationship, especially a theory of responsibility and total affirmation of individuals.

Japanese

Ikuko Noro, Associate Professor

General Summary

Suitability for patients of informed consent documents written in Japanese

A study was continued of the suitability for patients of informed consent documents written in Japanese. This year, I used the protocol analysis method to analyze comprehensibility and the uncertainty patients feel about informed consent documents.

Effects of physician-patient gender combination of physician and patient on medical communication

To examine the effects of the physician-patient gender combination on medical communication, physician-patient conversations were recorded at 3 hospitals and were analyzed with the Roter Interaction Analysis System.

Research Activities

Suitability for patients of informed consent documents written in Japanese A paper about the study was presented.

Noro I, Muramoto T. Incomprehensibility and uncertainty of informed consent docu-

ments-protocol analysis. Ars Linguistica (Linguistic Studies of Shizuoka 2010; 17: 95-113.

Effects of gender combination of physician and patient on medical communication I presented on the relationships among communication, gender, and patient satisfaction at the second annual conference of the Japanese Association of Health Communication held at Kyoto University in September 2010.

Publications

Noro I, Muramoto T. Incomprehensibility and uncertainty of informed consent documents-protocol analysis (in Japanese). *Ars Linguistica* 2010; **17:** 95–113.

Noro I, Oba R, Ota M. Medical communication and Japanese language education (in Japanese). *Nihon Health Communication Kenkyukai Zasshi* 2010; **1**: 13-7.

Mathematics

Katsuya Yokoi, Professor

Hiroshi Shiraishi, Assistant Professor

General Summary

1. To study dimension theory and topological dynamics

2. To consider the asymptotic behavior of estimators of optimal portfolios when the return processes are various stochastic processes.

Research Activities

1. We summarized results of the size of the chain recurrent set for generic maps. We studied omega-limit sets of nonautonomous discrete dynamical systems.

2. We discussed resampling procedures in the estimation of optimal portfolios when the financial returns are vector-valued non-Gaussian stationary processes. On the basis of the bootstrap method, we constructed an estimator of the lower tail of the estimation error. Moreover, we introduced the Estimation Error Efficient Portfolio, which considers the estimation error as the portfolio risk.

Publications

Shiraishi H. Resampling procedure to construct estimation error efficient portfolios for stationary returns of assets. *J Jpn Stat Soc* 2010; **40:** 189-206.

Reviews and Books

Yokoi K. The size of the chain recurrent set for generic maps (in Japanese). Suri Kaiseki Kenkyujo Kokyuroku 2011; **1728:** 79-83.

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 concerning how to make useful digital images and XML files of fifteenth century manuscripts, especially of the *Stonor Letters*. The results of this investigation were discussed in papers read at an international conference.

Fujii joined a project team to compile English textbooks for high school students. The team is aiming to receive approval for the textbooks from the Ministry of Education, Culture, Sports, Science and Technology. In addition, Fujii is working on teacher's manuals and exercise materials.

Research Activities

Ohara presented a paper at a session titled "Stylistic Consistency in the Letters of Thomas Betson" at the International Medieval Congress 2010 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 Archives of the United Kingdom and studied the *Stonor Letters*. Making use of the results of the study, Ohara continued his research on the graphemes of the *Stonor Letters*.

Fujii analyzed and collected authentic English materials to meet the needs of high school textbooks based on current teaching methodologies, theories, and research findings on learning English as a foreign language. These materials will be used to compile textbooks following the revised teaching guidelines set out by the Ministry of Education, Culture, Sports, Science and Technology. The first textbook of the series, *Communica-tion English I, II, and III*, will be used in high schools starting in April 2013.

First Foreign Languages

Yoshiaki Shirasaki, Professor

General Summery

1. I have continued educational activities for the purpose of verbal and nonverbal communication. These activities are also connected with the aim of developing moral and philosophical abilities in the field of intellectual relationships.

2. I have also studied Friedrich Schiller. This is a sort of paradigm innovation for this classic and ideological poet. Lang regarded Schiller to be a classical poet, and he was most productive writer of romantic operas. This is the reason I have continued this study.

School of Nursing

Basic Nursing

Sawako Haga, Professor 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 4 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, and a history of progressive muscular dystrophy wards.

Hanyu has been investigating the effects of physical assessment by nurses, the evidence of nursing skill, and stoma management.

Publications

Hirao M, Haga S, Kohiyama R (Tokyo Woman's Christian Univ). M.E. Reade: The pioneering educator of nurses in Meiji Japan. Jikeikai Med J 2010; 57: 113-9.

Reviews and Books

Kikuchi M. A history of progressive muscular dystrophy wards: the trends in the study of the treatment in PMD wards. *Tokyo Jikeikai Ika Daigaku Zasshi* 2010; **125:** 143-52.

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 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.

Publications

Takashima N, Oe M, Gokita K, Watabe S. Longitudinal changes in the stress of nursing students during a clinical practice in adult nursing: based on assessment of psychological and physiological stress (in Japanese). *Nippon Kango Kenkyu Gakkai Zasshi* 2010; **33:** 115-21.

Takashima N, Murata H, Watanabe C. The present status of perioperative nursing in the gastroenterological surgery under hospital-days shortening: national survey for recognition among outpatient administrators (in Japanese). Tokyo Jikeikai Ika Daigaku Zasshi 2010; **125:** 231-8.

Murata H, Inoue T. Clinical judgment of nurses

to continue noninvasive positive-pressure ventilation (NPPV) in patients with acute respiratory failure(in Japanese). *Nippon Critical Care Kango Gakkaishi* 2011; **7:** 36-44.

Reviews and Books

Imamura Y, Yotsu R, Shimizu H, Matsumoto K, Kitano M, Ota M, Takashima N. The vasculocardiosurgical patient's nursing (in Japanese). In: Kitajima M, Egawa K, editors. Surgical nursing in ditail. 8th ed. Tokyo: Igaku Shoin; 2011. p. 142-71.

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

Sakurai studied the psychology of families of elderly persons with dementia. We have been investigating the psychological conflicts of family caregivers for elderly persons with dementia.

Date has been investigating the health status and various factors influencing it among

middle-aged and elderly adults for the primary and secondary prevention of lifestylerelated disease from a comprehensive perspective, including nutrition, exercises, and rest.

Reviews and Books

Sakurai M. The philosophy of gerontological 15-20. nursing (in Japanese). Kango to Joho 2011; 18:

Mental Health and Psychiatric Nursing

Masashi Kawano, Professor

Junko Ishikawa, Assistant Professor

General Summary

We have revised contents and teaching-learning strategies for 4 years and finalized our revisions this year; in particular, we have tried to strengthen the relation between classes and practical training. By using role-playing teaching methods, which aim to realistically simulate patient situations, students can learn clinically applicable communication skills. We also used a video that made ourselves about practical clinical training in the hospital. It shows the role of nurses and the situation in psychiatric hospitals. The video also includes an important knowledge base. We have sought to carry out realitybased education, and we believe our program contents have been well developed.

Research Activities

Kawano and Ishikawa have collaborated in performing research about community mental health for children in Tokyo's Itabashi, Nerima, and Toshima wards. We are also in studying communication skills in undergraduate and graduate students, and in discourse analysis. We are also deeply interested in Human Caring. Kawano is interviewing discharged psychiatric patients who live in rural areas of Japan and Thailand and are analyzing the results to determine the issues that make farmer inpatients create difficult situations while living in the community.

Reviews and Books

Kawano M. Education and training for community psychiatric mental health (in Japanese). Seishinka 2010: 17: 291-4.

Child Nursing

Kiyo Hamanaka, Professor

Kayoko Cho, Associate Professor

General Summary

A summary of our first study was presented at a conference, which was of great importance to us. Since our second study was a large nationwide sample survey, the analyzed data should be presented so that the results can be put into practice. A summary of our third study was reported. I hope this will contribute to our future research.

Research Activities

Development and verification of an educational support program to continue working in child care

A summary of the development and verification of an educational support program performed before and after graduation was presented at a conference and published by a journal.

Cooperation of educational and medical staff to provide educational support for hospitalized children

Cooperation between medical and educational sites is particularly important to provide educational support for hospitalized children, including child with cancer. However, because few studies have examined this cooperation from a medical perspective, we began a national survey of chief nursing officers and nurse managers to investigate cooperation between medical and educational sites.

A study of establishing a nursing care unit and outpatient nursing practice performed as basic nursing education to promote children's health A 4-year study and its outcomes were summarized.

Publications

Arakawa M. Nurses' thoughts on the death of pediatric patients: Based on nurses' experiences with terminal care (in Japanese). *Tokyo Jyoshi Ika Daigaku Kango Gakkaishi* 2010; **5:** 11–9.

Reviews and Books

Hamanaka K, Hinuma C, Ohgi N, Nakamura Y, Ohya N, Kodama C. Development and verification of an educationional support program to continue working in child care: Development and practice (in Japanese). *Shouni Kango* 2010; **33**: 289-97.

Hinuma C, Hamanaka K, Ohgi N, Nakamura Y, Ohya N, Kodama C. Development and verification of an educationional support program to continue working in child care: Effect and problem (in Japanese). Shouni Kango 2010; **33**: 298-304.

Maternity Nursing

Kimiko Kayashima, Professor

Yasuko Hososaka, Assistant Professor

General Summary

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

Research Activities

Activities of midwives at youth clinics in Sweden and support for adolescents in Japan In June 2009, we performed an on-site investigation of youth clinics in Sweden, which are public facilities that aim to prevent sexually transmitted diseases and unwanted pregnancies in adolescents, primarily through the activities of midwives and social workers. Midwives engage in such activities as providing sexual health education, testing for sexually transmitted diseases, distributing condoms, and prescribing emergency contraception. More than 220 youth clinics have been established, and midwives have a wide range of duties and play a leading role in protecting the sexual health of youths. These results were presented at the 29th Annual Meeting of the Japan Society of Adolescentology, the First Board Meeting of the Healthy Parents and Children 21 initiative, and the lecture meeting of the Japanese Foundation for Sexual Health Medicine. We propose that similar facilities employing midwives should be established in Japan to protect the sexual health of youths.

Current research on the sexual function of pregnant women and nursing mothers and its trends

Although we investigated trends in nursing research on sexuality in fiscal year 2009, because few studies have investigated the sexuality of pregnant women and nursing mothers, we performed a literature review of the sexual function of pregnant women and nursing mothers and discussed its characteristics as well as approaches for supporting the sexual health of these women.

Microbiological, immunological, and nutritional safety of breast milk

The microbiological and immunological safety of breast milk was investigated for various storage and thawing methods using breast milk obtained from 20 adult nursing mothers 1 month postpartum and 2 samples of artificial milk for neonates. Bacteria content in breast milk was the highest in milk stored at room temperature followed by those stored frozen and refrigerated, indicating that bacterial counts are influenced by the storage method. In the immunological investigation, analysis was performed using immunoglobulin A and lipase as indicators. Lipase levels were found to decrease significantly for all thawing methods. For nutritional investigation, analysis was performed using glucose, total protein, total lipid, and total cholesterol. Total cholesterol levels were shown to decrease significantly following thawing in microwave ovens and hot water.

Establishment of a model for managing maternal body weight among pregnant women and underweight pregnant women to prevent infants from having low birth weights We have been performing a large-scale investigation since January 2010 at 27 obstetric facilities nationwide to clarify the effects on neonatal birth weight of the intrauterine environment, in particular, increases in the mother's body weight between prepregnancy and delivery.

Investigation of the degree of university instructor satisfaction and related factors

Preparations are being made to perform a study of the degree of satisfaction among university instructors engaged in practical training for maternity nursing, as well as relevant factors. The study will focus on maternity nursing instructors at 4-year universities in Japan, and preparations are being made to employ nursing university teachers' self-efficacy for nursing practice education, along with a simple questionnaire on occupational stress.

Publications

Hososaka Y, Nukita H, Kayashima K. The relationships between menstrual symptoms of female adolescents and causative physical and psychological factors. *Shishunkigaku* 2010; **28**: 227-38.

Reviews and Books

Kayashima K. Protect the sexual health in a

familiar region: Introduction of the Swedish Youth Clinic. *Shishunkigaku* 2011; **29:** 33-7.

Kayashima K. Protect the sexual health: through the activity of the midwives. In: For Healthcare worker & school nurse: Young person, sexual health and sex education. Tokyo: Sei no Kenko Igaku Zaidan; 2011. p. 5-12.

Community Health Nursing

Noriko Okuyama, Professor Ikuko Takahashi, Assistant Professor Junko Shimasawa, Associate Professor

General Summary

The faculty's research has been focused on: 1) public health nursing care to promote continued community life by mentally ill patients living at home and 2) infection control in the community.

Research Activities

Public health nursing care for mentally ill patients living at home The purpose of this study was to clarify the features of assistance provided by public health nursing care to promote continued community life by individuals with mentally ill patients living at home. In this study, such assistance was considered to be support that promoted continued life in the community of the mentally disabled individual in a manner suitable for that individual.

Infection control in the community

We do research on hand hygiene among care staff in care facilities for the aged. Hand hygiene is a basic measure to prevent infections. The purpose of this study was to obtain suggestions for the improvement of hand hygiene.

Home Care Nursing

Motoko Kita, Professor Hiroko Toyama, Assistant Professor Reiko Yoshida, Assistant Professor

General Summary

The subjects of educational research performed by the Department of Home Care Nursing included the use of mobile learning systems to help students effectively learn in home nursing training, methods to improve the effects of exercise-based home nursing classes, and other topics of interest to teachers.

Research Activities

A study of the use of mobile learning systems in clinical training

The Department of Home Care Nursing has effectively used an e-learning system for its clinical training in home nursing since 2006 because it is difficult for teachers to accompany students all the time to instruct and advise them. However, the insufficiency of educational materials and references for self-learning in training facilities has been an obstacle to effective learning by students during the training period. To address this problem, mobile computers have been installed in all of the facilities to allow anyone anywhere to obtain necessary information at any time. We developed a method based on mobile learning to respond to questions from students in real time and examined its effectiveness.

A study of the methods to improve the effects of exercise-based lessons in home nursing

Conventional exercise-based classes in home nursing have centered on role-playing using case examples to encourage students to develop their practical skills. In response to the recent revision of the curriculum and a marked increase in the time for home nursing lessons, we assessed the composition of lessons, case examples used in class, and approaches to learning taken by students to improve the quality of exercise-based home nursing classes.

Awareness of the right of nurses to prescribe drugs and problems among home-visit nurses and physicians involved in home nursing care

We performed interviews with home-visit nurses and physicians involved in home nursing to examine their awareness of the right of nurses to prescribe drugs and the status of their involvement in drug prescription in the home care setting. To grant nurses the right to prescribe drugs, both nurses and physicians are required to have clinical experience and expertise. The results show that granting of the right would provide advantages to both users and physicians.

Development of an index for assessing the stability of the life of families with elderly in need of nursing care

We are developing a scale to determine the stability of the life of families with elderly in need of nursing care on a continuing basis, as an index for assessing the outcomes of support provided for families. This study was performed to examine the applicability of the scale to care support provided for families.

The effects of a narrative intervention approach on anticipatory grief felt by family members who provide care until a terminally ill patient dies

People who provide nursing care at home for a family member sometimes suffer from a feeling of anticipatory grief. We performed a study of the families of terminally ill patients expected to die within 6 months to examine the effects of a narrative approach (an intervention) on anticipatory grief, including changes in its levels and characteristics.

Publications

Toyoyama H, Shimanouchi S. Grief care for bereaved families of deceased home-cared elderly in Japanese (in Japanese). *Kazoku Kandogaku Kenkyu* 2010; **15:** 18-29.

Reviews and Books

Hara M, Munemura Y, Kita M. The trend and issues of aromatherapy research in a nursing (in Japanese). Kango Jissen no Kagaku 2010; 35:

58-65.

Kita M, Ito K, Noguchi M, Akiyama M, Ogane H. Case study for clinical practice; Case study as a research (in Japanese). *Kango Jissen no Kagaku* 2011; **36**: 64-7.

Kita M. Nursing for a family caring for a frail elderly person (in Japanese). In: Kawashima M, editor. Gerontological nursing. Tokyo: Kango no Kagakusha; 2010. p. 269-92.

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