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Introduction

I am pleased to publish *Research Activities 2007*, which is a report on the scientific and educational activities at The Jikei University School of Medicine in 2007. In parallel with this English-language version, we publish a Japanese version each year. This report describes the research activities in the departments, institutes, and laboratories of the Medical Science Center at The Jikei University School of Medicine in 2007. In *Research Activities*, 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 department staff are limited to those above assistant professor. The titles of members of the academic staff of universities were changed at the beginning of the 2007 academic year at the order of the Ministry of Education, Culture, Sports, Science and Technology. The titles are now "professor," "associate professor" (formerly "assistant professor"), "assistant professor" (formerly "lecturer"), and "research associate" (formerly "assistant"). Research associates are engaged in education and research works and are recognized as members of the academic staff.

We also re-organized several departments, in particular, the departments of basic sciences. The Departments of Anatomy I and II were merged, so that the Department of Anatomy now has two professors. The department is responsible for the teaching of gross anatomy, neuroanatomy, histology, and embryology. The names of the Departments of Physiology I and II were changed to the Department of Molecular Physiology and the Department of Cell Physiology, respectively. The Departments of Microbiology I and II are now the Department of Bacteriology and the Department of Virology. The Department of Biochemistry I and II are now the Department of Biochemistry and the Department of Molecular Biology. Thus, numbered departments names are no longer used, and the activities of each department are more clearly described by the new names.

Research Activities is a short summary of the annual research works at The Jikei University School of Medicine. I hope that *Research Activities* is widely used by people outside our university as well as by our staff. In addition, this report will be used to evaluate The Jikei University School of Medicine on the basis of its research As President of The Jikei University School of Medicine, I have been activities. encouraging both traditional and innovative research, in particular, research that supports and is linked to the clinical activities of our university hospital. Findings of this traditional basic research will contribute to medicine in the future. I would like to emphasize the long-term importance of basic research. I have also been promoting research projects with a focus on topics in various areas, including molecular biology, and research employing highly advanced technology. Some results will contribute to the diagnosis and treatment of patients with refractory diseases. These projects have been partly supported by grants from the government (Ministry of Education, Culture, Sports, Science and Technology, and Ministry of Health, Labour and Welfare) and various private foundations. Advanced clinical research works of the clinical departments are mostly performed in the Medical Science Center at the university, because the

Medical Science Center is well equipped with various advanced instruments. We financially support the Medical Science Center and provide grants for young scientists to encourage their research activities. I hope that the research works supported by these grants will be continued. Some results of these projects are contained in this report.

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

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

Satoshi Kurihara President The Jikei University School of Medicine

February 2009

Continuing Medical Education Center The Continuing Medical Education Committee

Kunihiro Kurihara, Director Yutaka Suzuki Katsuyoshi Tojo Keizo Takagi Akihiko Ohno

General Summary

The Continuing Medical Education (CME) Center was established in 1982 to commemorate the centennial of The Jikei University and to support the education of doctors outside the university hospital. Registered members consist of alumni throughout Japan, members of the local medical association, and doctors who have been approved by the Jikei CME Center. Members are allowed to use the facilities (video, library) of the Center and other facilities (medical library, medical museum) of the university. A telephone service is available at all times. Members may also attend or participate in summer and monthly seminars sponsored by the Center, and in scientific meetings and conferences held by the department.

Activities

- Registered members: 253 (as of April 1, 2008) Members using the Center: 180 per year Telephone service: 124 cases
- 2. The 28th summer seminar was held on August 4, 2007. A total of 101 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. Each seminar was attended by 25 to 30 persons.
- 4. The "CME Center News" is mailed monthly to registered members.

Center for Medical Education

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

General Summary

The Office of Educational Development was founded in1999. Staff members were recruited from the School of Medicine. Our main interests were analysis of medical education reports published by the Ministry of Education, Culture, Sports, Science and technology (MECSST), Ministry of Health, Labor 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-service programs in undergraduate medical education. However, many improvements have been required in our undergraduate medical and nursing education, post-graduate 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 Post-graduate Clinical Training, and the Office of Educational Development. Furthermore, the secretariat was set up in the Center in April, 2006. The Office for Medical Education contributed to revisions of the undergraduate curriculum, to implementation of OSCEs in year 4 and 5, and to faculty development programs (writing multiple-choice questions, and rater training for OSCE). The Office of Nursing Education contributed to faculty development programs for nursing teachers (physical assessment training). The Office of Post-graduate Clinical Training contributed to management of residency program as active member of faculty, and revision of Clinico-Pathological Conference (CPC) for post-graduate year 1 and 2 doctors; and implementation of faculty development programs for attending doctors belonging to 4 attached hospitals. The office of Medical Development contributed to establish e-Learning system for student and health care providers in the community, and to implement several continuing learning courses (auscultation seminar) for district nurses in the community.

Research Activities

1. Promoting community-based medical education for undergraduate medical and nursing students, and developing continuing professional development programs for heath-care providers in the community: Our proposal was selected as a "Supporting grant for distinctive university educational 2007" by MECSST. We proposed an

extension of family medicine practice employed in year 5 medical education from 3 days to one week to the curriculum committee. As a trial, we supplied an auscultation seminar to district nurses who concerned in student practice "in-home care".

2. Developing a nurture program of clinical researcher for community young doctors: Our proposal was selected as a "Cultivating high quality health care professions according to social need 2007" program by MECSST. Twelve young doctors attended to this program, and started to learn about biological statistics, clinical epidemiology, and clinical research design which we supplied using e-Learning system and face-to-face workshops.

3. Risk-management and ethics Workshop in attached hospitals: We organized the workshops held at April (Nishishinbashi), May (Aoto), June (Daisan), July (Kashiwa), August and September (Nishishinbashi), October (Aoto), November (Nishishinbashi), December (Daisan), January (Kashiwa), and February (Nishishinbashi). A summary of repots written by attendance was published using an intranet home page.

4. Contribution to other institutions of higher education (Faculty Development lecture and workshop): Nippon Medical School (May), MECSST (July), Kanazawa Medical School (August), Saitama Medical School(August), Yamagata University (August), Kansai Health Science College (September), Kakogawa City Hospital (September), Tokyo Medical College (October and December), Kumamoto University (January), Naijyo Pharmaceutical College (January), OT/PT Association (January).

Publications

Nara N, Ito M, Goto H, Saito N, Tanaka Y, Tanabe M, Fukushima O, Horiuchi S. Comparative analysis of faculty development in Japanese medical schools from 2003 through 2005 (in Japanese). *Igaku Kyouiku* 2007; **38**: 275–8.

Noro I, Abe K, Matsushima M, Fukushima O, Kimura N. Effects of medical students' gender on communication during medical interviews (in Japanese). Igakukyouiku 2007; 39: 13-8.

Reviews

Fukushima O. OSCE in U.K. In: Ootaki J, editor. OSCE-theory and practice (in Japanese). Tokyo Shinohara Shuppannshinnsha; 2007.

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, the development and organization of neuronal networks were investigated to elucidate brain function and diseases by means of immunocytochemistry, electron microscopy, *in situ* hybridization histochemistry, single-cell tracer injection, and patch-clamp electrophysiology. Our primary interests are the architecture and dynamics of microcircuits. In gross anatomical research, the functional importance of variations of organ systems was studied using human cadavers and animals.

Research Activities

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

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

Neurons in the cNTS vary in cell size (50 to $450 \,\mu m^2$ in somal area) and other morphologic characteristics. For a more objective classification of cNTS neurons, their morphologic features were analyzed quantitatively on the basis of reconstructed biocytin-filled cells after whole-cell patch-clamp recording. According to the patterns of axonal

branching, cNTS cells could be classified into 2 groups: smaller cells (94.1 μ m² in mean somal area; range, 62–120 μ m²; n=22) and larger cells (245 μ m² in mean somal area; range, 142–411 μ m²; n=23). Extensive axonal arborization with numerous possible synaptic boutons was specifically associated with smaller neurons, whereas larger neurons possessed no or few axon collaterals; these findings suggest that smaller neurons serve as local-circuit neurons (or interneurons), whereas larger neurons are projection neurons. With regard to somatodendritic characteristics, the following correlations with cell size were found: smaller cells had larger form factors than did larger cells (P< 0.05), and larger neurons had more extensive dendritic arborization, expressed by total dendritic length (P<0.01) and 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, whereas large neurons convey the integrated information to other autonomic brain regions.

Postnatal development of GABAergic axon terminals in the rat cNTS

The proper function of the brain depends on a precise arrangement of excitatory and inhibitory synapses. Although the cNTS plays a pivotal role in cardiorespiratory reflexes, we know little about the local neural network in the cNTS. In the present study, we focused on GABAergic axon terminals and investigated postnatal changes in GABAergic synaptic organizations in the rat cNTS with immunocytochemical methods at both the light and electron microscopic levels. The counting of synaptic and nonsynaptic GABAergic axon terminals revealed that the number of GABAergic axon terminals in the cNTS was constant until the second postnatal week and that GABAergic axon terminals were reorganized at about postnatal day 10. Electron microscopic observation revealed that most GABAergic axon terminals had formed axosomatic synapses on neurons with smaller somata (smaller neurons) by postnatal days 2 to 4 but that the number of axosomatic synapses decreased considerably after postnatal day 8. Orphan GABAergic boutons were present specifically near somata of smaller neurons at postnatal day 10, and the number axodendritic synapses on thicker dendrites gradually decreased during postnatal development. These results show that GABAergic axon terminals detach from somata of smaller neurons during the second postnatal week. Such morphologic changes in axon terminals could cause changes in electrophysiological activity and might contribute to reorganization of the local network within the cNTS from the neonatal type to the adult type. These postnatal changes in the cNTS local network might be required for cardiorespiratory reflexes of the adult type.

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

Neural activity during critical periods could fine-tune functional synaptic connections. Activation of *N*-methyl-D-aspartate (NMDA) receptors is implicated in this process, and blockade leads to disruption of normal circuit formation. This phenomenon has been investigated in several neural systems, including the somatosensory system, but has not been observed in the visceral sensory system. Ultrastructural analysis of GABAergic synapses and electrophysiological analysis of inhibitory and excitatory postsynaptic currents of cNTS cells revealed that developmental changes in the synaptic organizations is blocked by MK-801, an NMDA receptor antagonist, when administered on postnatal days 5 to 8, 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 by electron microscopy and electrophysiology into 2 cell groups: small and large cells under far stronger excitatory and inhibitory influences, respectively. Blockade by MK-801 during the critical period might leave adult neurons wired in the undifferentiated synaptic networks, possibly preventing synapse elimination and subsequent stabilization of the proper wiring.

Glial coverage of the small cell somata in the rat cNTS during postnatal development Astrocytes are thought to be active participants in synaptic plasticity in the developing nervous system. Previous studies have suggested that the number of axosomatic synapses decrease on 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, glutamate-aspartate transporter (GLAST), and glutamate transporter 1 within the cNTS became more intensely stained as development proceeded. GLAST-positive structures encompassed calbindin-positive small cell somata after postnatal day 10. Electron microscopic observations indicated that astrocytic processes encompass the small cell somata, whereas the number of axosomatic synapses decreases as development proceeds. The timing for glial coverage of the small cell somata appears to be consistent with the decrease in axosomatic synapses on the small cells. These observations imply that astrocytes may participate actively in regulating the decrease in axosomatic synapses on small cells in the cNTS during postnatal development.

Publications

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Negishi Y, Kawai Y. Expression of neurocan in the rat nucleus of tractus solitarius during postnatal development. *Neurosci Res* 2007; **58**(Suppl): 206.

Department of Anatomy (Histology and Embryology)

Masataka Okabe, Professor Toshiaki Tachinbana, Assistant Professor Hisashi Hashimoto, Associate Professor Yasuyo Shigetani, 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

Molecular evidence that the lungs and the swim bladder are homologous organs The air-filled organs of land vertebrates and fish — the lungs and the swimbladder have long been postulated to be homologous organs. Both of them are air-filled sacs that derive from the digestive tract. On the other hand, the lungs are paired structures, whereas the swimbladder is a single sac. The lungs extend from the ventral side of the digestive tract, whereas the swimbladder extends from the dorsal side. Because of a lack of fossil evidence, determining whether the lungs and the swimbladder are in fact homologous organs has been difficult. We used an evolutionary developmental approach to address this issue. We found that TBX4, fibroblast growth factor (FGF) 10, and NKX2.1, key regulators of mammalian lung development, are also specifically expressed in the developing swimbladder of the zebrafish. Suppression of FGF 10 and TBX4 by antisense morpholino dramatically reduced swimbladder size. These observations show that the development of the swimbladder and that of the lungs employ the same evolutionarily conserved set of genes, strongly supporting the hypothesis that the two organs are indeed homologous.

Acquisition of a novel organ involved in regulation of calcium concentration in the blood during vertebrate evolution

During evolution, tetrapods obtained several new organs, including the parathyroid, metanephros, and placenta, all of which regulate the calcium concentration of the blood to allow tetrapods to live on land. Development of these organs requires transcription factors of the Gcm family. Here we show that Gcm2, which is a key regulator of parathyroid development in tetrapods, is expressed in the chloride cells of teleosts. The chloride cells are specialized cells scattered over the outer skin which are engaged in calcium-ion regulation, in addition to the gills, and are the evolutionary counterpart of the parathyroid gland. This reveals the general role of Gcm2 in the development of calcium-regulating organs, suggesting that changes in their expression pattern may have played an important role in the acquisition of new calcium-regulating organs in different

vertebrate taxa. To identify evolutionary changes in the genome responsible for the differential expression of Gcm2, we searched for enhancer elements driving the expression of Gcm2 in the chloride cells of zebrafish. We identified 2 enhancer regions around the Gcm2 locus which specifically drive expression in the chloride cells.

Differentiation of human mesenchymal stem cells into the collecting duct system in chicken embryos

We attempted to induce human mesenchymal stem cells (hMSCs) to differentiate into cells in the collecting duct system, a derivative of the ureteric bud (UB), by transplanting them into the chicken UB progenitor region. We showed by cell-lineage tracing experiments with DiI that common progenitors of the Wolffian duct (WD) and the UB were both present in the intermediate mesodermal cells of the neck-trunk boundary region of early embryos. We also showed that paired box gene (PAX) 2-expressing hMSCs transplanted into the UB progenitor region migrated caudally with the elongating WD and became integrated into the WD cells. However, hMSCs were rarely able to migrate to caudal end of the WD, where UBs are formed. Therefore, this year, we examined gene expression patterns of PAX2-expressing hMSCs under cell-culture conditions. The reverse transcriptase polymerase chain reaction showed that PAX2-expressing hMSCs did not express *LIM1*, a critical gene involved in WD/UB morphogenesis. The results also raise the possibility that—LIM1-expressing hMSCs differentiate into UB cells, then into cells in the collecting duct system.

Development of the mucosal vascular system in the distal colon of the fetal mouse The formation of crypts in the distal colon of the mouse was investigated in association with the development of vascular networks. For histological observation, $1-\mu m$ cross sections were made from the distal colons of 13- to 18-day fetal mice. The 3dimensional distribution of vascular networks in the organ was observed after the fetuses had been perfused with rhodamine isothiocyanate-labeled gelatin and immunostained for laminin to examine the boundary between the epithelium and the mesenchyme. At 13 days' gestation, the distal colon and its epithelium had formed a cylindrical tube, and a loose primary plexus of vessels had appeared in the mesenchyme. At 15 days' gestation, the caudal portion of the distal colon began to form crypts, and the vascular plexus constructed from only a few layers had separated from the boundary between the epithelium and the mesenchyme. As development proceeded, crypts were formed in the caudorostral direction. The developing crypts advanced into the vascular plexus, so that few vessels were situated in the mesenchyme between crypts. As the crypts elongated, these vessels formed a small plexus perpendicular to the primary plexus, while the primary plexus became monolayered and loosened. The new plexus was composed of ascending and traversing vessels, but the regular honeycomb-like plexuses around openings of crypts had not been established by 18 days' gestation. The vascular system and the crypts in the distal colon will require several more weeks after birth to be completed.

Study of skeletal muscle — specific manganese superoxide dismutase — deficient mice To investigate the pathological significance of oxidative stress in the skeletal muscle, we generated skeletal muscle — specific manganese superoxide dismutase — deficient mice. The mutant mice showed severe disturbances of physical activities but no atrophic changes in skeletal muscles. On histological and histochemical analyses, the mutant mice showed centralized nuclei in muscle fibers and selective loss of enzymatic activity in mitochondrial respiratory chain complexes. In addition, the mutant mice displayed increased oxidative DNA damage and the reduced ATP content in muscle.

Molecular mechanisms for development of the trigeminal ganglion

The trigeminal nerve is the largest cranial nerve, containing both sensory and motor neurons responsible primarily for sensation in the face and movement for mastication. The trigeminal ganglion comprises cells derived from two distinct origins: placode and neural crest cells. The mechanism of trigeminal ganglion development has been well-studied in the chick; however, the molecular mechanism remains unknown. We investigated the roles of secreted factors, such as FGF8, in trigeminal nerve development and performed expressed sequence tag (EST) analysis of unknown genes from head ectoderm. Implantation of FGF8-soaked beads underneath the trigeminal placode suppressed expression of *Brn3a*, the earliest trigeminal placode marker. Electroporation of the dominant negative type of *Sprouty2*, a repressor of FGF8, had the same effect. Genes for morphology and causable factors of diseases and related genes were isolated as EST clones, which may shed light upon the molecular mechanism that bridges the gap between the FGF8 pathway and *Brn3a*.

How to make figures and presentations that are friendly to color-blind persons

In scientific presentations and publications, color has become a significant vehicle for information and presentation effect. However, color perception varies greatly among individuals; in particular, red-green color blindness is found in 4% to 9% of males in various populations, a frequency comparable to that of the AB blood type. Thus inappropriate color choices can cause unexpected difficulty in understanding color figures. We are examining how color and color combinations are perceived by persons with various types of color vision, to develop a method for presenting color information that can convey maximal information to all persons, including those with color blindness. We introduced this method on our web site: http://www.nig.ac.jp/color

Publications

Ito T, Ohi S, Tachibana T, Takahara M, Hirabayashi T, Ishikawa H (Nihon Dental Univ), Kusakabe M (Tokyo Univ), Hashimoto H. Development of the mucosal vascular system in the distal colon of the fetal mouse. Anat Rec (Hoboken) 2008; 291: 65-73.

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stem cells in vitro. Hum Cell 2007; **20:** 111-8. Matsuno M¹, Kose H¹, Okabe M, Hiromi Y¹ ('Nath Inst Genet). TFIIH controls developmentallyregulated cell cycle progression as a holocomplex. Genes to cells: devoted to molecular & cellular mechanisms. *Genes Cells* 2007; **12:** 1289-300.

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Ohi S, Takahashi N, Hashimoto H, Tachibana T, Hirabayashi T, Sugiyama K, Yanaga K, Ishikawa H (Nihon Dental Univ). Establishment and characterization of an IGSK-2 cell line derived from ascitic fluid of recurrent hCG and somatostatin secreted adenocarcinoma of the stomach. *Hum Cell* 2007; **20:** 52-61.

Ninomiya K, Ohi S, Tabei I, Jin S, Tachibana T, Yamashita S, Yanaga K, Hashimoto H. Establishment and characterization of a cell line (BTIC) including HER-2-positive cells derived from pleural effusion of recurrent breast invasive ductal carcinoma, scirrhous type. *Hum Cell* 2007; **20:** 85-90.

Reviews

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Shimizu H (Nath Inst Genet), Okabe M. Evolutionary origin of autonomic regulation of physiological activities in vertebrate phyla. Journal of comparative physiology. A, Sensory, neural, and behavioral physiology 2007; **193:** 1013-9.

Department of Molecular Physiology

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

General Summary

Our efforts have been concentrated on clarifying the mechanism of skeletal muscle contraction.

Research Activities

Ion species affect water states in biological tissues

Magnetic resonance imaging (MRI) depicts cross sections of living humans on the basis of differential responses of water protons. To clarify the origin of the differential responses, we chose water molecules in the lattice space of skeletal muscle, where the milieu of the water molecules can be definitely described in relation to the structure of a sarcomere. Skeletal muscle fibers can be viewed as repeated sarcomeres. We observed the effects of ion species on the structure and function of skinned fibers, which lack a cell membrane to act as a diffusion barrier, to enable any artificial intracellular solutions to penetrate deep into the fiber. We found that the I⁻, which has the ability to salt out colloids, hydrates the fiber and suppresses maximal contracting force induced by Ca^{2+} . The results indicate that I⁻ potently induces the effects of general ionic strength, suggesting that the interfilament spacing of skeletal muscle is strongly affected by the state of water in the interfilament space.

Structure and function of the water within the cell revealed with MRI analysis

Our recent experiment with nuclear magnetic resonance of frog skeletal muscle revealed that tissue water is distinctly classified into 4 groups. From this point of view, MRI of skeletal muscle, the prostate (peripheral zone and prostate cancer), brain, and testis were reconsidered. With a 1.5-T MRI system, a single-slice 32-echo imaging pulse sequence was applied, and the obtained T_2 relaxation curves were analyzed with the Matlab software program (Mathworks, Inc., Natick, MA, USA).

We could separate T_2 relaxation curves obtained with MRI into 2 or 3 exponential components in the 4 tissues. Two exponential components in skeletal muscle obtained with MRI analysis coincided well with central 2 of 4 water components in frog skeletal muscle obtained with nuclear magnetic resonance analysis. The similarities between the testis and peripheral zone of the prostate and between brain and prostate cancers would represent histopathological features, probably cellular density.

The advantage of using an accelerometer to analyze the kinetics of athletes A general method to analyze the kinetics of performing athletes requires a complicated studio setup and an expensive video system. We attempted to overcome these obstacles through the use of tiny accelerometers placed on the athletes. We found that a combination of accelerometers and a portable digital recorder worked well with field athletes.

Effect of polyethyleneglycol on the myofilament lattice

Polyethyleneglycol narrows the lattice spacing of skinned skeletal muscle sarcomeres. Because the polyethyleneglycol molecule (molecular weight, 3,350) is several nanometers in size, a lattice spacing of 40 nm appears to be large enough for polyethyleneglycol to penetrate. To determine whether polyethyleneglycol penetrates the sarcomere, the specific gravity of myofibril suspensions from rabbit psoas muscle was measured in the presence or absence of polyethyleneglycol. If polyethyleneglycol does not penetrate into the sarcomere, the specific gravity of the supernatant after centrifugation of myofibril suspension is larger than the specific gravity of myofibril suspension. These measurements suggest that polyethyleneglycol diffuses into the sarcomere at half of the external concentration.

Structural change of mutant troponin related to hypertrophic cardiomyopathy

To clarify the molecular mechanism of troponin-related cardiomyopathy, a molecular dynamics study of the structure of troponin mutants related to familial hypertrophic cardiomyopathy was performed. Three different troponin T mutants related to hypertrophic cardiomyopathy — Glu244Asp, Lys247Arg, and Pro82Ser — were studied.

Dynamics was calculated by the use of the Amber software program (version 9). Model structures of troponin mutants were constructed by introducing the mutation to the crystal structure of human cardiac troponin (core region of the TIC complex) obtained from Protein Data Bank (ID number 1J1E). More than 6 trajectories of 1 ns were obtained for wild-type and mutant structures.

The electrostatic interaction between troponin I and troponin T, which linked the alpha helix of troponin T and troponin I in the wild type, was lost in the mutant. Furthermore, when a terminal residue of troponin I was pulled toward an actin molecule, mimicking the intramolecular force on activation, the observed structural changes differed between the mutant and the wild type. This difference would be involved in the development of cardiomyopathy.

Publications

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Department of Cell Physiology

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

Physiology of cardiac muscle

Alpha₁ (α_1)-adrenoceptor (AR) signaling plays important roles in the regulation of cardiac muscle functions under physiological and pathophysiological conditions. We investigated the effect of α_1 -AR subtype-specific stimulation on L-type Ca²⁺ current (I_{ca}) and the subtype-specific intracellular mechanisms for the regulation of L-type Ca²⁺ channels in isolated rat ventricular myocytes. We found that each receptor subtype couples with different G proteins, which produce opposite effects on I_{ca}. The coupling of α_{1A} -AR with Gq/11 potentiates I_{ca}. In contrast, α_{1B} -AR interacts with one of the pertussis toxin (PTX)-sensitive G proteins, Go, of which the β , γ -complex might directly inhibit channel activity. The coupling of specific α_1 -AR subtypes with PTX-sensitive G protein could exhibit the negative feedback response to α_1 -AR stimulation, and this mechanism would contribute to the protection of the heart from Ca²⁺ overload.

Leakage of Ca^{2+} from the sarcoplasmic reticulum is believed to be responsible for the decreased cardiac function in heart failure. We investigated how Ca^{2+} leakage is altered when the Ca^{2+} release channels are phosphorylated by protein kinase A, which is believed to be activated in cardiac failure. In saponin-treated skinned fibers, protein kinase A—dependent physophorylation of Ca^{2+} release channels slowly increases Ca^{2+} leakage.

The Frank-Starling law of the heart is the intrinsic mechanism for regulating contraction, and cardiac muscle can produce more active force through stretch (length-dependent activation). We focused on the role of troponin in length-dependent activation and exchanged cardiac troponin (cTn) with skeletal troponin (sTn) in skinned porcine left ventricular muscle. We found that quasicomplete reconstitution of thin filaments with fast sTn (prepared from rabbit psoas muscle) markedly attenuated the length-dependent increase in Ca²⁺ sensitivity. We examined the effect of MgADP (3 mM) on length-dependent activation. MgADP attenuated the length-dependent increase in the Ca²⁺ sensitivity of porcine skinned preparations with or without sTn. Inorganic phosphate (Pi, 20 mM), which decreased the Ca sensitivity, enhanced the length-dependent activation of the preparation with or without sTn. These results suggest that length-dependent activation is regulated through thin-filament activation. The higher fraction of active cross-bridges attenuates length-dependent activation, and the smaller fraction of

active cross-bridges enhances length-dependent activation. Thus, troponin is a key factor for modulating length-dependent activation through the state of thin filaments. Skinned cardiac fibers exhibit spontaneous oscillatory contraction (SPOC) over a broad range of intermediate activating conditions, namely, at a pCa of 6.0 to 5.0 (Ca-SPOC) or with the coexistence of MgADP and Pi under relaxing conditions (ADP-SPOC). We have reported that the period of sarcomeric oscillations in fibers correlates with that of resting heartbeat in various animal species. The present study was performed to analyze SPOC in single cardiomyocytes, because excitation-contraction coupling occurs at the cellular level. To enhance the quality of sarcomere length measurement, we used quantum dots conjugated with α -actinin antibodies to visualize the Z-line position during SPOC in a single sarcomere. We measured the period and amplitude of ADP- SPOC and Ca-SPOC at various sarcomere lengths and found that the period of sarcomeric oscillations is similar to that observed at the fiber level. These findings suggest that the intrinsic auto-oscillatory property of sarcomeres may contribute to myocardial beating *in vivo*.

A mouse model of dilated cardiomyopathy, in which mutant troponin T was knockedin, was used to investigate the mechanism of human dilated cardiomyopathy. The peak of the Ca^{2+} transient was larger and the force was smaller than in cardiac muscles of wild-type mouse. The results suggest a decrease in the Ca^{2+} sensitivity of the contractile elements. Variables measured with echocardiography showed cardiac dysfunction.

Ca²⁺ signaling in human parathyroid cells

This project was performed in collaboration with the Department of Nephrology and Hypertension. Cultured human parathyroid cells were loaded with a Ca²⁺-sensitive fluorescent probe, fluo-4, and placed on the stage of a Nipkow-type confocal microscope. Following exposure to a 150 mM K⁺ solution (containing 1.5 mM Ca²⁺) for more than 30 seconds, the subsequent application of a 150 mM K⁺ solution or a 3.0 mM Ca²⁺ solution (for 10 seconds) failed to evoke a transient increase in fluorescence (reflecting Ca²⁺ entry), suggesting that voltage-dependent Ca²⁺ channels are strongly inactivated by preceding long-lasting depolarization (manuscript under submission).

Because we had previously used a standard Dulbecco's modified Eagle's medium (containing 1.8 mM Ca²⁺) for parathyroid cell culture, we wondered whether a medium Ca²⁺ concentration less than 1.2 mM (the physiologic Ca²⁺ concentration) changes the activity of voltage-dependent Ca²⁺ entry. The amplitude of fluo-4 fluorescence evoked by a 150-mM K⁺ solution decreased in the following order: 1.8 mM Ca²⁺>1.2 mM Ca²⁺>0.9 mM Ca²⁺. The difference between 1.8 mM Ca²⁺ and 0.9 mM Ca²⁺ was highly significant (p<0.001), suggesting that an elevation of serum Ca²⁺ concentration somehow increases the expression of voltage-dependent Ca²⁺ channels, contributing to the negative-feedback regulation of parathyroid hormone secretion. We plan to investigate the correlation between the total charge carried by Ca²⁺ and the amplitude of fluo-4 fluorescence evoked by a variety of depolarizing pulses.

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Department of Biochemistry

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

Research Activities

Cancer research

1. To establish methods for the early diagnosis and treatment of cancer, several molecular properties of CD147 for cancer-cell — targeting has been evaluated. CD147, as an extracellular matrix metalloprotease inducer or basigin, is a transmembrane glycoprotein with 2 immunoglobulin-like domains. Using a murine monoclonal antibody against CD147 (MAb12C3) developed by Ohkawa, et al. in 1995, several studies were performed with clinical tumor specimens and demonstrated the significant correlations between prognosis and the expression levels of the CD147 protein in tumors and in patients with gynecologic malignancies (130 cases) or early hepatocellular carcinoma (22 cases). The expression levels of CD147 on the surfaces of tumor cells were significantly correlated with prognosis and with the malignant behavior of tumors, such as metastasis or invasion by tumor cells, even if small specimens had been obtained with fine-needle biopsy. The tumor-targeting ability via CD147 molecules on the surfaces of tumor cells was determined with tumor cells expressing CD147 at high levels and their CD147-knockdown sublines. The effects of an anti-CD147 antibodylabeled liposome (MAb12C3-liposome) encapsulating a glutathione-doxorubicin conjugate (GSH-DXR) on specific accumulation and cytotoxicity against CD147expressing human carcinoma cells were studied. After treatment of the cells with MAb12C3-liposome — encapsulated GSH-DXR for 2 hours, specific accumulation and cytotoxicity were observed in CD147-expressing cells but not in CD147-knockdown cells, suggesting that GSH-DXR-encapsulated MAb12C3-liposome would be an effective chemotherapeutic agent for CD147-expressing carcinoma cells. For the early detection, diagnosis, and treatment of cancer with ultrasound technology, the MAb12C3 antibody and its active Fab' fragments were coupled to ultrasound contrast agents, nano/micro In hepatoma cells grown in an *in-vitro* 3-dimensional culture, CD147-specific bubbles. bubbles can be detected with ultrasound devices and with infrared immunofluorescent devices.

2. The resistance of tumor cells to chemotherapeutic agents is a serious obstacle in cancer therapy. The GSH-DXR conjugate strongly inhibited the glutathione *S*-transferase (GST) activity of rat hepatoma AH66 cells. Treatment of the cells with GSH-DXR induced apoptosis, including caspase-3 activation, DNA fragmentation, and activation of c-Jun N-terminal kinase (JNK). Treatment of cells with GSH-DXR induced cytochrome c release from the mitochondria to the cytosol, followed by potent activation of caspase-3 and -9 with typical DNA fragmentation. JNK signaling is thought to be regulated by GST P1-1 via interaction with the C-terminals. In the present experiment, we found the C-terminal region of GST P1-1 binds to the JNK molecule and

that the active center of GST P1-1 plays important roles in the regulation of JNK enzyme activity. The findings suggest that inhibition of GST P1-1 activity by the binding of GSH-DXR to the active center of the enzyme activates JNK and induces apoptosis via the mitochondrial pathway in the cells. This study revealed a novel mechanism by which the enzyme activity of GST controls JNK activity.

3. Six epoxomicin-resistant cell lines were established. The epoxomicin-resistant cell lines are reliable tools 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. This year, the relation between expression of matrix metalloproteinases 14 and 2 and proteasomal inhibition was analyzed.

Other Research

1. With methods to purify and identify ubiquitinated proteins in biological materials, several ubiquitin-protein conjugates in Tris-saline—soluble and Tris-saline—insoluble 2% sodium dodecylsulfate (SDS)-soluble fractions were analyzed from cadmium-exposed human proximal tubular HK-2 cells and the brains of Niemann-Pick type C (NPC) disease (lipid storage disease with progressive neuronal death) model mice. The amino acid sequences of some of the purified ubiquitinated proteins were determined. The HK-2 cells exposed to cadmium at a concentration of 70 μ M (median lethal dose) showed a marked increase in ubiquitinated signal transducer and activator of transcription 6, interleukin-4-induced. In NPC mice brains, mean levels of the SDS-soluble ubiquitin-protein conjugates in the cerebrums of NPC (-/-) mice (aged 4 and 9 weeks) were significantly higher (increased 200%) than those of wild-type or heterozygous mice.

2. Regulatory mechanisms of transcriptional co-activator with PDZ-binding motif (TAZ) linked to the fibroblast growth factor (FGF)/receptor signaling, which plays an essential role in ossification, were determined with osteoblast-like MC3T3-E1 cells. We found that FGF-2, which inhibits bone mineralization and stimulates cell proliferation, reduced the TAZ protein expression level in MC3T3-E1 cells. The removal of FGF-2 from the culture medium reversed this reduction and restored the osteoblastic features of MC3T3-E1 cells. Furthermore, FGF-2-induced reduction of TAZ was blocked by an inhibitor specific for stress-activated protein kinase/JNK. These findings suggest that the expression of TAZ protein is involved in osteoblast proliferation and differentiation. 3. The radial-flow bioreactor (RFB) is a high-functioned 3-dimensional culture system that can be used for high-density culture that both maintains original cellular functions and mimics the architecture of human tissues. Several human cancer cell lines that were cultured in this system, rather than tumor tissues transplanted into nude mice, were used to prepare a well-organized artificial tumor tissue model in vitro. These results suggest that the RFB culture method is a useful and powerful system for improving and maintaining the conditions in acute liver failure and for assessing the efficacy and safety of newly synthesized drugs and biomaterials before application for clinical use. To evaluate the reduction of efficacy of anticancer agents against tumor

cells cultured in the RFB, gene and protein expressions in A431 tumor cells during culture were compared under physically different environments of 3-dimensional culture in the RFB, in 2-dimensional culture in a monolayer, and in nude mice.

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Department of Molecular Biology

Senya Matsufuji, Professor

General Summary

We have been studying the biological role and the regulatory mechanism of polyamines by antizyme (AZ). Polyamines, such as putrescine, spermidine, and spermine, are biogenic polycathions that are present in cells of all types. Polyamines are essential for cellular growth and regulate the function of various biomolecules. Polyamines also induce a regulatory protein called AZ. AZ accelerates the degradation of ornithine decarboxylase (ODC), the key enzyme of polyamine biosynthesis, and inhibits the cellular uptake of polyamines. The induction of AZ by polyamines involves a unique mechanism, translational frameshifting. AZ is evolutionally conserved in a wide range of eukaryotes and, in mammals, 3 paralogues (AZ1, AZ2, and AZ3) have diverged.

Research Activities

Study of the physiological roles of AZ1 in knockout mice

AZ1 is a major form of AZ expressed systemically. The phenotype of homozygous AZ1 knockout mice is partial embryonic death. Embryos lacking AZ1 show decreased numbers of hematopoietic cells in the liver. With the colony forming assay, burstforming unit-erythroid (BFU-e) was decreased in each secondary hematopoietic organ, namely the aorta-gonado-mesonephros (AGM) region, the fetal liver, and the adult administration of an ODC inhibitor, bone marrow. Maternal αdifluoromethylornithine (DFMO), prevented the hematopoietic disturbance in embryos lacking AZ1. Further analysis demonstrated that maternal administration of DFMO during the AGM period prevented the decrease in BFU-e in the fetal liver and the adult bone marrow. In addition, pretreatment with putrescine of floating cells from the AGM region of wild-type mice resulted in a decrease of BFU-e, but the addition of putrescine to soft agar medium during the colony-forming assay did not have this effect. These results suggest that exposure of early hematopoietic cells in the AGM region to a high concentration of putrescine is a major cause of the hematopoietic disturbances in AZ1 knockout mice.

Previous studies have shown that AZ1 knockout mice that survive until adulthood have body weights lower than those of their wild-type littermates. We examined the mechanism of this lean phenotype. AZ1 knockout mice born after maternal administration of DFMO showed body weights equal to those of their wild-type littermates at birth, but after 9 to 12 weeks they weighed less than their wild-type littermates. Blood hemoglobin levels were identical in AZ1 knockout mice and wild-type controls both at birth and at 9 to 12 weeks. Body fat mass measured with X-ray computed tomography (CT) was significantly lower in AZ1 knockout mice than in wild-type controls 12 to 14 weeks after birth, and the difference increased thereafter. Polyamine synthesis of adult AZ1 knockout mice was significantly elevated, and spermidine/spermine N^1 acetyltransferase (SSAT) activity was 50% as high as in wild-type controls. Transgenic mice overproducing SSAT reportedly have a lean phenotype due to overconsumption of acetyl-CoA. A similar mechanism may account in part for the low body fat mass in AZ1 knockout mice. However, because the increase in SSAT activity in AZ1 knockout mice was much smaller than that in SSAT transgenic mice, other mechanisms for the body fat loss are likely involved.

Analysis of AZ2-interacting proteins

AZ2, the second form of AZ, is expressed throughout the body, as is AZ1, but at much lower levels. To search for AZ2-specific functions, we screened AZ2-interacting proteins from mouse brain and liver cDNA libraries with a yeast two-hybrid system and discovered cerebellar degeneration-related protein 2 (CDR2) as an AZ2-binding protein. CDR2 binds to AZ2 but not to AZ1 or AZ3. Although the function of CDR2 is unknown, it reportedly binds to c-Myc through its leucine zipper domain. This year we examined the interactions among AZ2, CDR2, and c-Myc with pull-down assays in human-derived 293F cells. The interaction between AZ2 and CDR2 was decreased with c-Myc expression, whereas the interaction between c-Myc and CDR2 was decreased with AZ2 expression; this result suggests competitive binding of AZ2 and c-Myc to CDR2. Next, the effect of AZ2 on the stability of CDR2 was analyzed in transfected 293 cells to assess the biological significance of the interaction. We postulated that AZ2 could destabilize CDR2, as does its main target, ODC. However, CDR2 was stabilized with co-transfection of AZ2. Finally, the subcellular localization of AZ2 and CDR2 in Neuro2a cells was examined using fusion proteins with enhanced cyan or yellow fluorescent proteins (ECFP, EYFP). When separately expressed, AZ2 was mainly localized in the nucleus, and CDR2 was localized in the cytoplasm. Co-expression of the 2 molecules changed the localization of AZ2, which became co-localized with CDR2 in cytoplasm, indicating their interaction in the cells.

In a related project, we screened AZ2-interacting proteins from mouse kidney cDNA libraries with a yeast two-hybrid system and obtained 75 candidate clones. We rescreened these candidates under stricter conditions and obtained 8 molecules. Pull-down assay was performed to confirm the specificity of interaction between AZ2 and these molecules and demonstrated that 3 of the 8 proteins interact only with AZ2 and that 2 proteins interact with both AZ1 and AZ2. Next, these proteins were tagged with EYFP and expressed in cells either alone or with ECFP-tagged AZ2. Observation with fluorescent microscopy revealed that AZ2 had a markedly different subcellular localization and was now co-localized with candidate proteins when co-expressed with 2 of the candidate proteins.

Study of RNA-binding proteins that bind to the pseudoknot structure of AZ mRNA The mRNA of AZ has a pseudoknot structure as a signal for polyamine-dependent translational frameshifting, but the precise mechanism of this regulation has not been clear. We hypothesized that a protein that binds to the pseudoknot structure is involved in the translational frameshifting; to test this hypothesis we surveyed such RNA-binding proteins with the UV-crosslinking method. A protein of 34 kDa that specifically bound to a mutant version of AZ1 mRNA pseudoknot was detected in human-derived 293F cells and was successfully purified using the pseudoknot RNA as an affinity ligand. Analysis with the peptide mass fingerprint method identified 2 candidates, namely heterogeneous ribonucleoprotein (hnRNP) A1 and a related protein, hnRNP A1 like (hnRNP A1L). The cDNAs of these proteins were introduced into 293F cells with the dual luciferase reporter gene system to measure their effects on AZ1 translational frameshifting. Dual luciferase assay revealed that hnRNP A1L doubled the frequency of frameshifting, whereas hnRNP A1 had no effect. Stimulatory effects of hnRNP A1L were observed, however, both on the reporter genes with the wild-type pseudoknot and with the mutant pseudoknot. We are now confirming the binding of these proteins to the pseudoknot structure and their effects on frameshifting in vivo by preparing recombinant hnRNP A1 and hnRNP A1L proteins from *Escherichia coli*.

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Department of Pharmacology

Masahiro Kawamura, Professor Naofumi Kimura, Professor Noboru Nakamichi, Assistant Professor Haruhisa Nishi, Assistant Professor Seiji Hori, Professor Kazuo Takano, Associate Professor Yuji Ohno, Assistant Professor

General Summary

Our main interests are the physiological roles of nucleotide receptors and the mechanisms of regulation of intracellular Ca²⁺ concentration as an intracellular messenger in several kinds of cells, including bovine adrenocortical fasciculate cells (BAFCs), 3T3-L1 preadipocytes, Madin-Darby canine kidney (MDCK) cells, and brain astrocytes. We are also interested in the convulsant activity of quinolones, the anti-inflammatory activity of methylxanthines including theophylline, the neural mechanisms of vagal inspiration-promoting reflex, the visualization of respiratory neural activities, the effect of the urocortin family on the cardiovascular system, and clinical pharmacology.

Research Activities

Cross-talk between ACTH receptors and purinergic receptors in BAFCs

Extracellular adenosine triphosphate (ATP) and uridine triphosphate (UTP) bind to plasma membrane P2 receptors to regulate several cell functions in many kinds of cells. P2 receptors are divided into 2 families: the ligand-gated P2X and the G proteincoupled P2Y. The P2Y family has at least 8 subfamilies. We have previously reported that BAFCs contain Gq-protein-coupled P2Y₂. Both extracellular ATP and UTP bind to P2Y₂ to stimulate Ca²⁺ influx via IP₃ production from the extracellular space. ACTH is a physiological stimulator of adrenocortrical steroidogenesis via production of cyclic adenosine monophosphate (cAMP). We found that BAFCs express at least 3 types of adenylyl cyclase, the Ca²⁺-calmodulin-potentiated types I and III, and the β -subunit-potentiated type II. We have reported that ATP and UTP potentiate both ACTH-induced steroidogenesis and cAMP production. Our findings suggest that the Ca²⁺-influx pathway or the β subunit of Gq protein (which links to P2Y₂) or both are involved in the cross-talk between the ACTH receptor and P2Y₂. Results under our experimental conditions suggest that the β subunit of Gq protein, but not the Ca²⁺ pathway, participates in the event.

Study of purinergic receptors and steroidogenesis in a human adrenocortical tumor cell line (H295R cells)

The interaction between purinergic receptors, especially the $P2Y_2$, and ACTH receptors, was described above. However, because of the Japanese Public Health ordinance for the prevention of bovine spongiform encephalopathy, obtaining fresh bovine adrenal glands is now impossible. Therefore, preparing large quantities of fresh BAFCs has become difficult. Cells of the H295R line respond to ACTH and produce cortisol (a

physiological glucocorticoid) via a cAMP-dependent system. Thus, this cell line could be an excellent model for adrenocortical cells. We obtained this cell line and established cell culture conditions. We found that H295R cells express functional $P2Y_2$ and that extracellular ATP stimulates cortisol production.

Study of the intracellular Ca²⁺ dynamics

 Ca^{2+} is an important regulator in many cellular functions. Therefore, we studied intracellular Ca^{2+} dynamics and its physiological functions in BAFCs, 3T3-L1 preadipocytes, MDCK cells, and brain astrocytes through the use of samples loaded with fluorescent calcium indicators (fura-2 and fluo-4).

1. Store-operated Ca²⁺ entry in BAFCs

Store-operated Ca^{2+} entry (SOCE), *i.e.*, Ca^{2+} entry triggered by the depletion of Ca^{2+} in the endoplasmic reticulum (ER), plays an important physiological role in nonexcitable cells. Both Gq-protein-coupled receptor agonists stimulate IP₃ production followed by Ca^{2+} release from the ER, and cyclopiazonic acid, a sarcoplasmic/endoplasmic reticulum Ca^{2+} (SERCA) pump inhibitor, inhibits Ca^{2+} reuptake and depresses the luminal Ca^{2+} concentration. Then, Ca^{2+} enters from the extracellular space through the activation of SOCE. Three hypotheses for the mechanism of SOCE have been proposed: 1) the conformational coupling model, 2) the exocytotic model, and 3) the diffusible messenger model. We have previously reported that BAFCs have a steroidogenesis-coupled SOCE system that is closely related to the actin network and that the conformational coupling model is the most prominent model in BAFCs. However, the identity of the plasma membrane SOCE channel in BAFCs is still unclear. One candidate for the SOCE channel is the transient receptor potential protein (TRP), especially the TRPC subtype. Our results, however, show that TRPC is not the SOCE channel in BAFCs.

2. SOCE in 3T3-L1 preadipocytes

3T3-L1 preadipocytes differentiate to adipose cells under the controlled culture conditions. Intracellular Ca²⁺ mobilization is an important factor in adipodifferentiation. The early stage of adipodifferentiation in 3T3-L1 preadipocytes is reportedly inhibited by Ca^{2+} . Therefore, we studied the mechanism of Ca^{2+} influx in 3T3-L1 preadipocytes. We found that the cells have an SOCE system that is activated by prostaglandin (PG) $F2\alpha$ via Gq-protein-coupled PG receptors and thapsigargin, a SERCA pump inhibitor. 3T3-L1 preadipocytes reportedly have PG receptors and secrete PGF2 α . These characteristics suggest the possible involvement, in an autocrine/paracrine fashion, of PGF2 α in the adipodifferentiation of 3T3-L1 preadipocytes. The SOCE induced by PGF2 α and thapsigargin was not abolished by treatment with the actin cytoskeleton modifying agents cytochalasin D and calyculin A. The results suggest that the SOCE model in 3T3-L1 preadipocytes is the diffusible-messenger model. The cells expressed 4 TRPC subtypes. However, these TRPCs did not participate in SOCE in 3T3-L1 preadipocytes under our experimental conditions. Recently, STIM1, an ER membrane protein, and Orail, a plasma membrane protein, have been suggested to be involved in SOCE. We found STIM1 in 3T3-L1 preadipocytes.

3. Intracellular Ca²⁺ dynamics in MDCK cells

Autosomal dominant polycystic kidney disease is a common genetic disorder. Although the pathogenesis of this disease remains unclear, recent observations suggest that abnormalities of primary cilia play an important role in renal cyst formation. Primary cilia respond to mechanical bending by flow, and increase in the intracellular Ca^{2+} . MDCK cells are often used to study the mechanism of polycystic kidney formation. Therefore, we studied intracellular Ca^{2+} dynamics in MDCK cells. We found that spontaneous calcium oscillations occurred in the cells, even in the absence of primary cilia and extracellular Ca^{2+} . Spontaneous calcium oscillations in MDCK cells are suggested to be initiated by Ca^{2+} release from ryanodine/IP3-sensitive intracellular Ca^{2+} store, an ER. The frequency of calcium oscillations was enhanced by extracellular ATP. Our results suggest that Ca^{2+} release from the ER is an important process and that ATP is a physiological factor modifying spontaneous calcium oscillations in MDCK cells.

4. Astrocytic calcium oscillations in rat hippocampal slice cultures

An increasing body of evidence indicates that ATP mediates glia-to/from-neuron signaling in the central nervous system (CNS) network. Extracellular ATP has various target proteins in both glia and neurons in the CNS, including ATP receptors and adenosine receptors, which are activated after converted extracellular ATP is rapidly converted to adenosine by ectoenzymes. Activation of purinergic receptors regulates astrocytic calcium oscillations, which are spontaneous events in astrocytes. However, the mechanism is not fully understood. We studied the role of extracellular ATP in the regulation of spontaneous astrocytic calcium oscillation in fluo-4-loaded rat hippocampal slice cultures using calcium imaging. We have reported that the activation of adenosine A_{2B} receptors modulates astrocytic calcium oscillations through acceleration of extracellular ATP breakdown into adenosine in the hippocampus of the rat.

Convulsant activity of new quinolones

New quinolones have been reported to have potent convulsant activity. We compared the convulsant activity of new quinolones in young mice and adult mice. New quinolones induced convulsions in a dose-dependent manner in young mice, and the ED_{50} values in young mice were similar to those in adult mice. Furthermore, co-administration with anti-inflammatory drugs enhanced the convulsant activity of new quinolones in both young mice and adult mice. The interaction of new quinolones and anti-inflammatory drugs did not differ between young mice and adult mice.

Anti-inflammatory activity of theophylline

Because theophylline has been reported to have anti-inflammatory activity, we studied the effects of theophylline and other methylxanthines on carrageenan-induced edema in rats. Theophylline and caffeine inhibited carrageenan-induced edema in rats. The inhibition of carrageenan-induced edema was suppressed by pretreatment with mefepristone or aminogluthetimide. These results suggest that theophylline has antiinflammatory activity that is involved in the glucocorticoid-glucocorticoid receptor

system.

Visualization of the spatiotemporal pattern of respiratory neural activities in the isolated frog brainstem

We visualized the spatiotemporal activity of respiratory-related neurons in the frog by means of an isolated brainstem spinal cord preparation. We recorded optical signals from the ventral surface of the medulla with a voltage-sensitive dye and calculated cross-correlations with the integrated respiratory activity of the trigeminal nerve. Lung-burst-related depolarizing optical signals were observed bilaterally as longitudinal columns in the ventrolateral medulla between the levels of the trigeminal and hypoglossal rootlets, mostly caudal to the vagal nerve rootlet. However, we could not differentiate between neurons involved in rhythm generation and motoneurons. Extracellular recordings of respiration-related neurons verified the optically identified area. Strychnine disrupted the spatiotemporal organization of optical signals, although trigeminal periodic bursts persisted. We conclude that the pattern generator, but not the rhythm generator, of the lung burst in the frog involves glycinergic mechanisms and lies in longitudinal columns in the reticular formation of the ventrolateral medulla. (Collaboration with the Department of Physiology, Hyogo College of Medicine, Hyogo, and the Department of Medicine, Keio University Tsukigase Rehabilitation Center).

Neurophysiological and neuropharmacological studies on the central respiratory mechanism

1. Neural mechanisms of the vagal inspiration-promoting reflex and involvement of P2X receptors in nucleus of the solitary tract

To understand how respiratory movements are optimized in response to changes in internal and external environments, the research group for mammalian respiratory function has been studying neural mechanisms underlying various types of respiratory reflexes in mammals (in collaboration with the Laboratory of Neurophysiology, Department of Neuroscience). We have demonstrated that a local microinjection of pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS), a P2X receptor antagonist, and kynurenic acid, a glutamate receptor antagonist, into the caudal nucleus of the solitary tract (NTS) of the anesthetized rabbit. The inspiration-promotion reflex of Hering-Breuer was attenuated by PPADS injection, and the inspiration-suppression reflex of Hering-Breuer was inhibited by kynurenic acid. Histological analysis of the site of action of PPADS showed that the marked reduction of the vagal inspiratory promotion reflex occurs most often when the site of PPADS injection, as identified with staining, contained the caudal part of the lateral NTS. This result argues clearly for a pivotal role of P2X receptors in the caudal NTS in the inspiration-promoting responses activated by decreased pulmonary stretch-receptor inputs.

Studies of the effects of cardiovascular regulatory substances on rat cardiomyocyte function

We have studied the effects of cardiovascular regulatory factors and agents on primary cultured neonatal rat cardiomyocytes. However, some factors and agents have little

effect on isolated cardiomyocytes but have more prominent effects on cardiomyocytes in the diseased human heart or in animal models. In pathological conditions of the heart, the ratio of noncardiac myocytes to cardiomyocytes increases. Therefore, the cross-talk between cardiomyocytes and noncardiac myocytes should be clarified. We developed a cardiomyocyte/noncardiac myocyte co-culture system using rat neonatal cardiocytes to address the difficulties of evaluating the effects of cardiovascular agents on cardiomyocytes and reported the fundamental data of this co-culture system. In addition, we investigated the intracellular dynamics of urocortin and its related peptides using HL-1 cardiomyocytes and some agents affecting rat cardiomyocytes.

Study of blood lactate level to evaluate the athletic performance

Many studies have examined muscle performance by measuring blood lactate concentrations during high-intensity exercise in athletes, and blood lactate is considered the metabolic variable that best reflects the capability of muscles for athletic performance. However, few studies have been performed in nonathletes, and the blood lactate concentration has not been recognized as a standard variable. Therefore, we investigated the effect of a 10-minute light bicycle load on the blood lactate concentration during the athletic performance of nonathletes. The blood lactate concentration during loading in nonathletes increased more quickly and markedly than in athletes but by 30 minutes after loading had returned to the pre-exercise level. In conclusion, even with light exercise, athletic performance can be evaluated by measuring blood lactate concentrations in a time-dependent manner.

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Department of Pathology

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

General Summary

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

Research Activities

Hepatology

We continued a histopathological study of nonalcoholic steatohepatitis. The additional cases of nonalcoholic steatohepatitis were examined. The results supported the previous conclusion that the pericellular fibrosis developing around the central veins and its terminal branches in one lobule expanded and connected in a band-like fashion to similar areas of fibrosis in adjacent lobules and that the fibrous band encircled the intact portal tract incompletely.

We studied chronic viral hepatitis histopathologically. Three-dimensional observation demonstrated that in the early stage the portal branches forming the framework of the liver lobules were damaged and were lost in part, with the consequences of portal-to-central fibrous bridging, subsequent widespread and progressive damage to the portal branches, distortion of the lobular architecture, and regenerative nodule formation. These changes indicate the process of self-assembly of histologic structures in a thermodynamic nonequilibrial system.

Damage and repair of the DNA of hepatocytes caused by oxidative stress in liver cirrhosis and chronic hepatitis were investigated immunohistichemically. The expression of 2 markers of oxidative stress, 8-hydroxy-2'-deoxyguanosine and thymidine glycol, were examined. The results showed that 8-hydroxy-2'-deoxyguanosine was expressed mainly by hepatocytes and that thymidine glycol was strongly positive in various cells, including hepatocytes, epithelial cells of bile ducts, endothelial cells, sinusoidal cells, and lymphocytes.

The development and distribution of lymphatic channels were examined immunohistochemically using D_240 and CD34 in normal liver, chronic hepatitis, and liver cirrhosis. Lymphatic channels increased in number and tended to be dilated in chronic hepatitis and liver cirrhosis. These lymphatic channels were distributed preferentially in the capsule and subcapsular portal areas. The development of lymphatic channels was considered to be correlated with fibrosis.

The 3-dimensional structure of cystic lesions in polycystic disease of the liver was examined with serial histologic sections. The results indicated that proliferating ductules changed to von Meyenburg complexes, which continued to grow and became large cysts lined with ductular epithelium. Some von Meyenburg complexes were connected to preexisting bile ducts, but others were not. These cystic structures were nourished by the arterial blood supply.

Renal pathology

We continued our histologic examination of mitochondrial nephropathy. Tubular cells with cytoplasmic granular swelling were removed by means of laser microdissection. Electron microscopic examination revealed abnormal mitochondria, which were considered to have caused histologic changes of the cells.

Fifteen kidneys obtained at autopsy after bone marrow transplantation were subjected to histologic examination. Thrombotic microangiopathy was detected in 9 cases. However, the relationship between thrombotic microangiopathy and calcineurin inhibitor was obscure.

Histologic examination in cases of chronic rejection of renal transplants revealed peritubular capillaritis and thickening of the capillary basement membrane. Endothelial cells positive for caveolin-1 increased in number. These findings were correlated with the severity of chronic rejection.

New criteria for the prognosis of IgA nephropathy were established on the basis of the results of histologic examination of specimens collected nationwide.

A new disease concept, glomerular deposition disease, was proposed.

Histologic examination of biopsy specimens obtained from transplanted kidneys indicated that patchy tubular injury was correlated with local circulatory disturbance of the transplanted kidney. Also it might be an unknown marker suggesting vascular rejection.

Specimens of renal cell carcinoma collected in the department were re-evaluated histologically with revised general rules for clinical and pathological studies of renal cell carcinoma. We found that cystic renal cell carcinoma with cystic lesions comprising more than 50% of the cut surface of the kidney had a better prognosis than did cases with cystic lesions comprising less than 50% of the cut surface.

Gastrointestinal pathology

Risk factors for metastasis were examined in 136 surgically resected specimens of colorectal cancer with submucosal invasion. Lymphatic channels were immunohisto-chemically 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 lymphatic invasion and blood vessel invasion were closely related to lymphatic metastasis.

Gynecologic pathology

Uterine cervical adenocarcinoma was examined with special reference to human papil-

loma virus (HPV) infection. We found that that the development of cervical adenocarcinoma with common histologic subtypes was closely related to HPV infection. On the other hand, cervical adenocarcinoma with uncommon subtypes were unrelated to HPV infection.

The pathogenesis of ovarian borderline malignancy derived from the müllerian duct was studied. The relationship of reserve cells of cervical gland-like cells to the development of ovarian borderline tumor was noted.

The distribution of reserve cells of cervical gland-like cells was examined in ovarian borderline tumor of müllerian type.

Urogenital pathology

Clinicopathological studies of prostatic carcinoma were continued. Histologic changes during the treatment of prostatic cancer were examined, and the response to treatment was evaluated.

Frequency of loss of heterozygosity was studied in prostatic cancers with special reference to tumor volume.

Soft-tissue tumor

Borderline vascular tumors, including composite hemangioendothelioma and perivscular epithelioid cell tumors, were examined clinicopathologically. The histologic characteristics and prognosis of epithelioid trophoblastic tumor was studied.

Oncology

Whole base sequences of mitochondrial DNA and genomic instability-related genes were examined with the polymerase chain reaction — single-strand conformation polymorphism method in gastric, colonic, and renal cancers. The results indicated that genomic instability was not involved in the development of these cancers.

The loss of heterozygosity in the short arm of chromosome 8 was analyzed in 86 specimens of liver cell cancer obtained at autopsy. The 8p22-23 loci were frequently lost. This finding indicates that the loss of these loci play important roles in calcinogenesis and, especially, in the metastasis of liver cell cancer.

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Department of Virology

Kazuhiro Kondo, Professor

Takaaki Ohashi, Assistant Professor

General Summary

Human herpesvirus (HHV) 6 belongs to the human β -herpesvirus subfamily, which consists of human cytomegalovirus, HHV-6, and HHV-7. Both HHV-6 and HHV-7 belong to the Roseolovirus genus of the β -herpesviruses, and the HHV-6 species are divided into 2 variants: HHV-6A and HHV-6B. β -herpesviruses can establish a lifelong latent infection of the host and are reactivated frequently, and some evidence suggests that the molecular mechanisms of viral latency and reactivation are common among these viruses. We are studying the molecular mechanisms of latency and pathogenesis of β -herpesviruses. Additionally, we are trying to apply these viruses to studies of the mechanism of fatigue and as viral vectors for gene therapy.

Research Activities

Mood disorders, chronic fatigue syndrome, and herpesvirus

Chronic fatigue syndrome (CFS) is a disease of unknown etiology whose chief complaint is severe fatigue. The prominence of the acute onset of illness, the persistent symptoms consistent with a viral infection, the increased titers of viral antibodies, and the enhanced activity of interferon-induced enzymes suggest viruses play a role in CFS. In other words, CFS might be a type of "postinfectious fatigue syndrome" following any viral infection. The viruses evaluated to date include enteroviruses, retroviruses, and HHVs.

CFS is a disease that lasts far longer than postinfectious fatigue. Therefore, the infection causing CFS might be a latent infection with an HHV. Of the HHVs, HHV-6 is the most promising candidate for a CFS-associated virus. Because an unusual latent infection with HHV-6 may cause CFS, the study of latent infection is important for determining the cause of CFS.

Several lines of evidence suggest that latent HHV-6 infection in the brain is involved in some neurological diseases, such as recurrent febrile convulsion, multiple sclerosis, and encephalitis. However, the site of HHV-6 latency in the brain has not been identified. To determine the mechanism by which CFS is contracted, we attempted to identify a special latent HHV-6 infection state called the "intermediate phase," described above. In this phase, several types of HHV-6 latency-associated protein corresponding to EBNAs might be expressed. This intermediate stage is observed in the first phase when HHV-6 commences reactivation but is completely different from reactivation in that no virus is produced. To examine the relationship between disease and latent infection proteins, whose manifestation is promoted in the intermediate stage, titers of antibodies to cells in which latent infection proteins were well manifested were examined in the serum of patients with CFS. This examination revealed that about 40% of patients with

CFS showed antibody reactions to intermediate-phase HHV-6 latent infection, whereas healthy subjects showed virtually no reaction.

β -Herpesviruses latency/reactivation

Fatigue is a familiar problem of daily life. Many people who are under stress suffer from various kinds of fatigue. However, scientific and medical studies relating to fatigue have been inadequate, and few studies have employed clear, quantitative, and objective means for examining fatigue, which is subjective manifestation.

To date, muscle fatigue (exercise fatigue) has mainly been studied as a representative example of fatigue. The main indicator in muscle fatigue is an increase in the production of lactic acid in muscles. However, lactic acid is an important energy source for the central nervous system, and the theory that lactic acid inhibits muscle activity has been disproven. In addition, during muscle fatigue, pyruvic acid increases and pH decreases in body fluids. These phenomena are indeed observed when a certain stress, i.e., a load to muscle (exercise load), is given; however, fatigue differs from local muscle exhaustion and is considered to be a broader and larger physiological phenomenon.

Both HHV-6 and HHV-7 are almost universally acquired by 2 to 3 years of age. These viruses belong to the β -herpesvirus subfamily and are closely related to each other, as shown by biological and molecular analyses. They establish life-long latency, a hallmark of herpesviruses, reactivate frequently, and are shed in saliva.

To investigate viral reactivation, we have identified the latency-associated transcripts of HHV-6 and have clarified, in part, the mechanism of HHV-6 reactivation. HHV-6 establishes latency in macrophages and maintains a fairly stable intermediate stage between latency and reactivation, which is induced by 2 or more factors.

HHV-6 can reactivate in immunosuppressed patients; however, the relationship between immunosuppression and the induction of reactivation is unclear. To identify the factor or factors of HHV-6 reactivation, we have studied the association with HHV-6 reactivation and work-induced fatigue in healthy adults. Immune strength is thought to deteriorate when humans are fatigued, and virus infection reflects this deterioration of immune strength. However, the relationship between fatigue and virus infection in humans remains unclear.

HHV-6 DNA was detected in 88% of subjects engaged in moderately excessive work due to long working hours and other factors (the first test day). In contrast, HHV-6 DNA was detected in 23.8% of subjects immediately after a holiday (the second test day). These results show that HHV-6 is significantly reactivated on exertion. These results led to the discovery that HHV-6 DNA expressed in saliva through the reactivation of HHV-6 is a fatigue biomarker (a biological index factor) that varies according to fatigue. Accordingly, a simple and objective method was developed for assessing the degree of fatigue by detecting HHV-6 DNA released into saliva as a result of the reactivation of HHV-6.

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

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

Accumulation of knowledge and various technological advances in molecular biology and molecular genetics have greatly contributed to the recent progress in life sciences, providing much information about various biological phenomena. Research and development in various fields of life science are ongoing, with an emphasis on the analysis of gene functions. These activities have led to the development of techniques and vectors for introducing isolated genes into cells and individual living organisms. Virus vectors have advantages over other known vectors in introducing a foreign gene into a cell for protein expression. The central idea underlying gene transfer with virus vectors is to introduce a foreign gene into an infected cell and transform the cell with the foreign gene under the control of promoter sequences, taking advantage of the infectious capacity of the virus (productive infection, latent infection, and abortive infection).

In particular, HHV-6 and HHV-7 have drawn much interest as candidate virus vectors for gene therapy, because infection with these viruses produces mild symptoms. Herpesvirus, and HHV-6 and HHV-7 in particular, have certain advantages as recombinant viruses and recombinant virus vectors, which include low pathogenicity, ease of gene introduction into blood cells, such as T cells and macrophages, and the ability to introduce large genes. However, no method has been available for producing recombinant viruses and recombinant virus vectors from HHV-6 or HHV-7. Factors complicating the recombination of HHV-6 and HHV-7, other than technical factors, are the characteristics of the HHV-6 and HHV-7 genes.

We have identified the dispensable genes of HHV-6 and HHV-7 and have reported the establishment of recombinant HHV-6 and HHV-7. The dispensable locus of HHV-6 was approximately 8.4 kbp, and that of HHV-7 was approximately 7.3 kbp; these sites are useful for insertion of large genes. The exogenous nucleotide sequence may encode at least 1 type of substance, including artificial bacterial chromosomes, cytokine genes, ribozymes, interference RNA, immunological co-stimulator molecules, signal transduction molecules, enzymes, and chemical attractants. Furthermore, exogenous nucleotide sequences may be used for the gene therapy of mammals. Gene therapy might be used to prevent the infection of compromised cells by human immunodeficiency virus and for the immunotherapy of cancer.

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Department of Bacteriology

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

General Summary

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

Research Activities

A biofilm destruction factor secreted by an indigenous bacterium inhibits pathogenic bacterial colonization

Although indigenous bacteria are generally considered to inhibit pathogen colonization, complex host-microbe and microbe-microbe interactions have made it difficult to understand in detail the mechanisms that might be involved. In this study, we demonstrate that Esp, a protein belonging to the serine protease family which is secreted by the indigenous bacterium *S. epidermidis*, inhibits *S. aureus* nasal colonization via biofilm destruction. *S. aureus* is the main pathogen colonizing the nasal cavity in humans. Both in vitro and epidemiological studies have demonstrated that *S. epidermidis* strains can be categorized into inhibitory and noninhibitory types on the basis of their inhibition of *S. aureus* colonization. Furthermore, *S. aureus* was significantly less prevalent when the inhibitory *S. epidermidis* type was present within the nasal cavity. The molecule responsible for the inhibitory activity was Esp. This molecule destroyed the biofilms of *S. aureus*, including those of methicillin- and vancomycin-resistant strains, by disrupting the intercellular matrix but not by bactericidal activity.

Inhibition of endothelial IL-8 production and neutrophil transmigration by S. aureus β -hemolysin

The innate immune system plays a crucial role in the host response to infection with *S. aureus*, and leukocyte migration is a key event in host defense against bacterial infection. The endothelium plays an important role in neutrophil recruitment through modulation of the expression of cell adhesion molecules and cytokines, such as IL-8, which is a potent chemoattractant and activator of neutrophils.

We have previously reported that *S. aureus* secretes a factor that suppresses IL-8 production by human endothelial cells. Here we isolated the factor inhibiting IL-8 production from the supernatant and identified it as staphylococcal β -hemolysin. This

protein is an enzyme that specifically cleaves sphingomyelin, the major sphingolipid in membranes, and is highly hemolytic for sheep erythrocytes. However, little is known about the action of β -hemolysin on host cells.

 β -hemolysin reduced IL-8 production without cyotoxicity to endothelial cells. Pretreatment with β -hemolysin decreased the expression of both IL-8 mRNA and protein induced by tumor necrosis (TNF)- α . The migration of neutrophils across TNF- α — activated endothelium was also inhibited by β -hemolysin. β -hemolysin reduced VCAM-1 expression but not ICAM-1 expression in activated endothelial cells. β -hemolysin did not inhibit activation of nuclear factor κ B but did inhibit activation of extracellular signal — regulated kinase. These results show that β -hemolysin produced by *S. aureus* interferes with inflammatory signaling in endothelial cells and may help *S. aureus* to evade host immune responses.

Interaction between fibroblasts and S. aureus at different growth phases

A clinical isolate *S. aureus* OK11 was used. Staphylococci grown in brain heart infusion broth at 37°C for 2 hours (exponential phase) and 18 hours (stationary phase) were treated with purified fibronectin to examine the participation of fibronectin in the ingestion of bacteria by L929 fibroblasts. The bacterial numbers in each L929 fibroblast were counted under a light microscope. We have shown that the exponential phase staphylococci expressed a large amount of FnBP on their surfaces, whereas stationary phase cells did not. L929 fibroblasts that had a network of fibronectin on their surfaces ingested a large number of bacteria in the exponential phase but ingested fewer bacteria after they had been treated with fibronectin. This result suggests that fibronectin is an adhesin. Because L929 fibroblasts with fibronectin could ingest more staphylococci in the stationary phase than could L929 fibroblasts without fibronectin, fibroblasts might have other adhesins involved with bacterial ingestion.

Contribution of FnBPB to fibronectin-mediated colonization in S. aureus infection

To colonize host tissues and organs *S. aureus* has a variety of adhesions that bind to extracellular matrix proteins or plasma proteins. Of these adhesions, FnBPs are thought to be the most important for interacting with host cells, such as endothelial cells, epithelial cells, fibroblasts, and macrophages. As we have already reported using the *fnbA* mutant strain derived from the parental SH1000 strain, FnBPA is important for infection by *S. aureus* both *in vitro* and *in vivo* because it allows the effective colonization of host tissues.

On the other hand, the role of FnBPB in infection is poorly understood. The DNA sequence of *fnbB* suggests that FnBPB lacks 1 or 2 of the fibronectin-binding domains and a fibrinogen-binding domain. Therefore, FnBPB might be less important for the establishment of infection than is FnBPA. However, our results described above showed the complete reduction of colonization in both *in vitro* and *in vivo* experiments. These results suggest that FnBPB has no importance in infection, although it is expressed at levels equal to those of FnBPA, and that FnBPA and B cooperate with one another for exhibiting their functions. To determine whether these possibilities are true, we are planning to obtain an *fnbB*-deficient mutant and *fnbA/fnbB* double-deficient mutant

from the SH1000 strain.

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Department of Public Health and Environmental Medicine

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

General Summary

Our major research projects in the 2007 academic year focused on: 1) the genotoxic effects of vitamin E and its derivatives on micronucleus induction; 2) the genotoxic effects of indium chloride on micronucleus induction; 3) the comet assay for detecting DNA damage; 4) DNA damage by exposure to electromagnetic fields (EMFs); 5) effects of zinc deficiency on the induction of chromosome aberrations; 6) oxygen-induced oxidative stress; 7) the genotoxicity of methylenedioxymethamphetamine (MDMA) as an illegal drug of abuse; 8) methods of medical informatics education and evidence-based medicine (EBM); and 9) the risk of decompression sickness.

Research Activities

Experimental medicine

1. A genotoxicity study of several derivatives of vitamin E that react as antioxidants (a collaborative study with Kyoritsu Pharmaceutical University)

A genotoxicity study of several derivatives of vitamin E was performed. Chinese hamster lung (CHL/IU) cells were exposed separately to both vitamin E derivatives and vitamin E. The number of chromosomal aberrations and micronucleus frequencies was comparable in the vitamin E derivatives and vitamin E.

2. Effects of indium chloride on micronucleus induction

Recently, concerns have been raised about the possible toxic effects of indium compounds used to diagnose myelopoiesis and of ceramics used in the production of transparent conductive films for flat-panel liquid crystal and plasma displays. The frequency of micronuclei induced by indium chloride increased in *in-vivo* micronucleus tests in BALB/c mice. Indium chloride also induced micronuclei on the in-vitro micronucleus test, but negative results were obtained on chromosome aberration testing with CHL/IU cells.

3. A possible mechanism for the enhancement by co-exposure to static magnetic fields of micronucleus formation by mutagens

We have previously found that co-exposure to static magnetic fields (SMFs) increases the frequency of micronuclei induction by several mutagens. In the present study, it became clear that the increase in the frequency of micronuclei induction by co-exposure to SMFs and chemicals was blocked by pretreatment with the antioxidant ascorbic acid. Moreover, an increase in 8-hydroxy-deoxyguanosine in bone marrow cells was observed after exposure to SMFs. These results suggest that exposure to SMFs enhances the reaction of mutagen-related radicals and DNA.

4. DNA damage by exposure to EMFs

We studied the effect of the co-exposure to EMFs and bleomycin on DNA damage using the comet assay. DNA damage induced by bleomycin in brain cells was increased by co-exposure to EMFs (50 Hz, 10 mT, 6 hours). Tempol, a radical scavenger, partially suppressed the DNA damage induced by bleomycin.

5. Effects of zinc deficiency on the induction of chromosome aberrations

An estimated 30% of Japanese have zinc deficiency. We studied whether zinc deficiency increases the risk of carcinogenesis. We found that the frequency of micronuclei increased 4 weeks after zinc-deficient food was first given. In addition, 8-hydroxy-deoxyguanosine was induced in bone marrow cells. These results suggest that an oxidative state is aggravated by zinc deficiency and induces micronuclei.

6. Oxidative stress by breathing oxygen in rat neonates

To clarify the carcinogenicity of target organs by oxygen exposure during the neonatal period, we studied oxidative DNA damage in animals.

7. Genotoxicity of MDMA as an illegal drug of abuse

The illegal use of MDMA has recently become a social problem. Orally ingested MDMA reacts with nitrites in the stomach and is metabolized into N-nitroso-MDMA. We synthesized N-nitroso-MDMA and studied its genotoxicity using the micronucleus and chromosomal aberration tests. The genotoxicity of N-nitroso-MDMA was observed with both tests.

Epidemiology, EBM, investigation, and medical informatics

1. Epidemiology, EBM, and medical informatics

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. A survey of risk of decompression sickness

To prevent decompression sickness in caisson workers and commercial divers, we have used the ultrasonic bubble detection technique to study the risk of decompression sickness under high-pressure mixed gas.

Publications

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Department of Forensic Medicine

Kimiharu Iwadate, Professor

Kenji Fukui, Assistant Professor

General Summary

Our main research projects in 2007 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; identification by DNA analysis of remains of the war dead; the objective evaluation of the limits of DNA typing based on the intensity of ninhydrin treatment; age and skewed X chromosome inactivation in autopsy specimens; and quantitative analyses of medicines and poisonous substances in forensic autopsy cases.

Research Activities

Forensic pathology

1. Sudden unexpected infant death due to milk aspiration

To examine differences in the pathological findings of the lung between intravital and postmortem cases of milk aspiration, an experimental study using a murine model was carried out. The results of immunostaining with an anti-human α lactalbumin antibody and an anti-cow whey antibody indicated that milk aspirated intravitally was distributed in the lungs more widely than was milk injected postmortem. However, the difference between them was not significant enough that they could be clearly distinguished by pathological findings alone.

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 the diagnosis of death by drowning. From the preserved blood samples of 30 cases of drowning in fresh water, specific DNA fragments of *Aeromonas sobria*, a most common aquatic bacterium, were examined with the polymerase chain reaction (PCR). The DNA fragments of the bacterium were detected with first PCR or nested PCR in 15 of 30 cases.

DNA analysis

1. Identification of the remains of war dead by means of DNA analysis

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

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

Shed epithelial cells on a sheet of paper were stained with ninhydrin reagent, and DNA

typing was performed. We studied the relationship between the intensity of the purple staining after ninhydrin treatment and the limits of DNA typing as mitochondrial DNA polymorphisms, and we attempted to perform an objective evaluation to determine the target of the stained area for DNA analysis.

3. Age and skewed X chromosome inactivation in autopsy specimens

We studied the association of age and skewed X chromosome inactivation in autopsy specimens from women. Two X chromosomes were differentiated by means of methylation-sensitive enzymes and the PCR for the human androgen receptor gene. A weak correlation was found between age and the frequency of skewed X chromosome inactivation, and we attempted to apply this finding to forensic age estimation.

Forensic toxicology

1. Quantitative analyses of medicines and poisonous substances

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

Publications

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Department of Tropical Medicine

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

General Summary

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

Research Activities

Malaria and mast cells

Malaria is one of the most serious tropical diseases. We have proposed a hypothesis that pericapillary mast cells are a major factor in the pathogenesis of malaria. We have demonstrated that mast cells release a large amount of tumor necrosis factor, resulting in protection from *Plasmodium* in innate and acquired immunity. Recent studies have shown that mast cells produce vascular endothelial growth factor (VEGF), which activates macrophages and induces adhesion molecules in blood vessels. We have examined the effects of VEGF in murine malaria. VEGF levels in the serum increased after *Plasmodium berghei* infection. Parasitemia was decreased by administration of VEGF to infected mice. On the other hand, treatment with an anti-VEGF antibody increased parasitemia. These findings suggest a protective role of VEGF in malaria. In the next experiment, mast-cell-deficient mice were given injections of cultured mast cells from normal mice and were then infected. These mice had higher protective activity and VEGF levels than did untreated control mice. These results indicate that VEGF from mast cells participates in the protection against malaria and that mast cells are competent for immune protection.

Expulsion mechanisms of gastrointestinal parasites

Interleukin (IL)-4 and IL-13, both of which are derived from T helper type 2 cells, are involved in the expulsion of gastrointestinal nematodes. Experiments in mice have shown that the signal operates through non-bone marrow-derived cells in the small intestine, suggesting that the direct effector cells are not immune cells but cells of other types, such as epithelial cells and smooth muscle cells. Both cytokines are involved in the water/ion regulation of epithelial cells and the contraction of intestinal smooth muscle cells. These functions are also regulated by nerve systems. The selective α 2-adrenoceptor agonist domitor inhibits sodium and water secretion into the lumen and the contraction of smooth muscle cells. The expulsion of adult *Nippostrongylus brasiliensis* from the small intestine in mice was suppressed by treatment with domitor. The expulsion was restored following treatment with a selective α 2-adrenoceptor antagonist. In fact, treatment with domitor inhibited mucus secretion and peristaltic motion of the small intestine in infected mice. Adult worms in domitor-treated mice were driven from the mucosa to the lumen and stayed there without being expelled. These findings indicate that the suppression of worm expulsion by domitor was due to inhibition of peristaltic motion. These results also suggest that responses of the nervous system are also involved in immune-mediated expulsion of adult *N. brasiliensis* in mice. Protection against secondary infection by eggs of *Vampirolepis nana* develops within several days after primary infection in mice. We attempted to identify the type of cell involved in this protection by using monoclonal antibodies to lymphocyte antigens. When an anti-CD3 antibody was administrated 1 day before primary or secondary infection, the protection against secondary infection was inhibited. However, administration of an anti-CD4 antibody 1 day before primary infection. Administration of an anti-CD8 antibody had no effect. These results suggest that the protection depends on CD4⁺ lymphocytes for the induction phase and CD4⁻CD8⁻ lymphocytes for the effector phase and indicate the participation of a novel subset of lymphocytes for mucosal immune responses.

Analysis of serine proteases, which mediate the excystation and metacytic development of Entamoeba

The functions of cysteine proteases involved in the pathogenicity and differentiation of Entamoeba histolytica have been demonstrated, but little is known about the functions of serine proteases. This study examined the involvement of serine proteases in amoebic excystation and metacystic development using *Entamoeba invadens* as the model of *E*. *histolytica*. Four serine-protease inhibitors — phenylmethylsulphonyl fluoride (PMSF), aminoethylbenzenesulphonyl fluoride, tosylphenylalanylchloromethyl ketone and dichloroisoproterenol — given at different concentrations decreased the number of metacystic amoebae in a dose-dependent manner but did not affect the survival of cysts. PMSF also inhibited the development of metacystic amoebae. PMSF effectively inhibited serine protease activity in cystic lysates. These data demonstrate the involvement of serine proteases in amoebic excystation and development. E. invadens has each two types of enzymes of serine protease family member S28 and S9 in the genome databases. The real-time reverse transcriptase polymerase chain reaction revealed that the mRNA expression levels of these serine proteases 5 hours after induction of excystation were higher than those before induction, in which an increase in expression of one type of the S9 enzyme was most significant. These results indicate that serine proteases mediate the excystation and metacystic development of *Entamoeba* and that serine protease mRNA levels in amoeba cysts increase after induction of excystation, especially that of the one type of S9.

Protein analysis of amoeba isolates with a liquid chromatography/tandem mass spectrometry system

Comprehensive analysis of proteins from amoeba isolates was performed with a liquid chromatography-electrospray ionization/tandem mass spectrometry system. Ten strains of cultured *E. histolytica* (including 5 domestic isolates) and 2 strains of a nonpathogenic amoeba, *Entamoeba dispar*, were used. Detected proteins were searched for with

Mascot Search, and the amount of protein was estimated from the exponentially modified protein abundance index (emPAI) for comparison. Actophorin and pyruvate phosphate dikinase, which were detected and identified in all isolates, showed a difference in the emPAI values according to the different protein expression patterns among strains. Alcohol dehydrogenase 3 and coactosin were detected and identified in all *E. histolytica* isolates but not in 2 *E. dispar* strains. In contrast, 40S ribosomal protein S18 and peroxiredoxin were detected and identified in the 2 *E. dispar* strains but not in the *E. histolytica* isolates. These results suggest that the present system is effective for detecting and identifying proteins of amoeba isolates with their expression levels and for distinguishing between *E. histolytica* and *E. dispar*.

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Department of Laboratory Medicine

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

General Summary

Research projects in our department in 2007 were concerned with clinical physiology, clinical microbiology, clinical chemistry, clinical biochemistry, hematology, cardiology, clinical cell biology, and clinical psychiatry. Research achievements in each division are described below.

Research Activities

Clinical physiology

The present study investigated the effects of acid-base disturbance on blood lactate kinetics, the onset of blood lactate accumulation (OBLA), the lactate threshold (LT), and the ventilation threshold (VT) during incremental cycle exercise. Nine healthy male volunteers participated in this study. Subjects were orally given NH_4Cl , $NaHCO_3$, and NaCl at doses of 1.87 mM/kg body weight. Exercise was started at a load of 80 W and was subsequently increased by 10 W per minute using the ramp method until voluntary exhaustion 60 minutes after NH_4Cl , $NaHCO_3$, and NaCl were administered. Both LT and OBLA were significantly higher in subjects who received acidotic treatment and were lower in subjects who received alkalotic treatment than in subjects who received control treatment. Acid-base disturbance induced differences in the blood lactate response to the same exercise load and affected LT, OBLA, and VT. Care must be taken when using LT, OBLA, and VT as indices of aerobic capacity in some patients with acid-base disorders, such as those who are undergoing hemodialysis, are obese, or have diabetes.

Clinical microbiology

The DNA diagnosis of clinical fungal infection was established with mycotic universal sequencing and applied to tissues of patient with mycosis. The minimum inhibitory concentration of fosphomycin with ciprofloxacin for multidrug-resistant *Pseudomonas aeruginosa* was measured with an oxygen potential electrode system and applied to the patient. The dioxin chemical structure was proposed for anti-inflamatory, antiproliferative agents versus the resistance mechanisms of the thermophilic bacterium *Bacillus midousuji*.

Clinical chemistry

To investigate how liver disease alters serum glycated proteins as markers of diabetic

control, we studied serum glycated albumin (GA), hemoglobin Alc (Alc), and, especially, the GA/Alc ratio in 255 patients with an alanine aminotransferase level greater than 35 IU/L in comparison with those in 829 patients with type 2 diabetes mellitus in a cross-sectional manner. The 255 patients with liver disease were divided into groups of 69 patients with biopsy-proven liver cirrhosis, 66 patients with chronic hepatitis, and 120 patients with fatty liver diagnosed with abdominal echography. The mean GA/A1c ratio (\pm SD) was significantly higher (p<0.0001) in patients with cirrhosis (3.71 ± 1.03) than in patients with hepatitis (3.03 ± 0.45) or diabetes (3.05 ± 0.42) , whereas the mean GA/A1c ratio in patients with fatter liver (2.74 ± 0.31) was significantly lower (p < 0.0001) than that in patients with diabetes. In patients with cirrhosis the GA/Alc ratio increased significantly depending upon reductions in the serum albumin level or the platelet count or both. The GA/Alc ratio was significantly correlated with other laboratory variables, such as serum levels of albumin, cholinesterase, and total cholesterol, and was weakly correlated with the blood hemoglobin level. We also followed the serum levels of GA and A1c and the GA/A1c ratio for 13 months (blood was collected 5 times) in 18 patients enrolled in this study. The coefficient of variation of the GA/Alc ratio was smaller than that of GA or Alc. The receiver operating characteristic curve of the GA/Alc ratio for patients with cirrhosis versus those with fatty liver was the most reliable between the 4 groups, and the cut-off value for patients with cirrhosis versus those with fatty liver was 2.94. These results suggest that the GA/Alc ratio could be a useful marker for the differential diagnosis of patients with abnormal serum levels of alanine aminotransferase.

Clinical biochemistry

We performed several research studies and obtained the following results.

1. Current issues of low-density lipoprotein homogenous assay were highlighted by lipoprotein analysis with a method of high-performance liquid chromatography we developed.

2. Supervised, regular exercise training for 4 months can increased adiponectin levels, but exercise for 2 months produces less significant increases.

3. Evaluation of very low density lipoprotein cholesterol is useful for monitoring lipoprotein amelioration.

4. Subanalysis of the Japanese Investigation of Kinetic Evaluation in Hypertensive Event And Remodeling Treatment Study showed that treatment with an angiotensin II receptor antagonist can prevent cardiovascular events in Japanese women.

Hematology

Abnormal chromosome patterns in multiple myeloma were compared according to gender and age. The hypodiploid pattern was most frequently found and was followed by hyper, pseudo, and tri/tetra patterns. The hypodiploid pattern was commonly found in younger patients, whereas the hyperdiploid pattern was frequently detected in older patients. In older patients, changes of +15 and -13 were most common and were followed by +11, +19, and +3 in both genders and in older patients. Deletion of a sex chromosome was found in 56% of men and 40% of women and was common

in younger patients. Band 14q32 was most common and was followed by 11q13, 1p22, and 8q24. There were many characteristics of gender and age, such as band 11q13 in men, and band 8p11 in younger patients. These results indicate several characteristics in modal number, imbalance, complexity, and breakpoints that were related to the gender and age of patients. These characteristics should be recognized when the karyotype of multiple myeloma is evaluated.

Cardiology

We are studying catheter intervention for atrial fibrillation. To eliminate atrial fibrillation, segmental ostial catheter ablation (SOCA) was performed to electrically isolate the pulmonary veins from the left atrium. Our aim is to improve the method of SOCA. This year we showed the efficacy of 2 new methods of SOCA. One method is segmental pulmonary vein antrum isolation using the large lasso catheter, and the other method is reducing the recurrence of atrial fibrillation by eliminating ATP-induced transient venous reconduction.

Clinical cell biology

- 1. Immunohistochemical study of lecithin retinol acyltransferase in hepatic stellate cells in liver diseases
- 2. Examinations of hepatic metabolism and the toxicity of drugs by means of a mini-bioartificial liver and stable isotope compounds
- 3. Development of an implant-type bioartificial liver
- 4. ¹³C-glucose breath test for diagnosis of insulin resistance
- 5. Ultrasound molecular imaging for diagnosing small cancers

6. Plasma examination for the transforming growth factor β activating reaction to diagnose liver damage

Clinical psychiatry

We reported on the effects of psychotropic drugs (antidepressants or antipsychotics) on electroencephalography (EEG) from the viewpoint of the radioreceptor binding (histamine H1) profile.

A retrospective study is under way to clarify the clinical significance of the 6-Hz spike and wave on EEG. We have continued to investigate epileptic seizures and chronological EEG changes in mentally handicapped patients. We reported on a patient with epilepsy and a psychiatric disorder who underwent temporal lobectomy and was successfully treated and reported on a patient with abnormal behavior during sleep who likely had epilepsy. Moreover, we examined premonitory signs and symptoms as epileptic prodromes.

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Department of Internal Medicine Division of Gastroenterology and Hepatology

Hisao Tajiri, Professor Ichiro Takagi, Professor Hirokazu Nishino, Associate Professor Tateki Yamane, Assistant Professor Michiko Negishi, Assistant Professor Tomohisa Ishikawa, Assistant Professor Kazuhiko Koike, Assistant Professor Kiyotaka Fujise, Professor Yoshio Aizawa, Associate Professor Hisato Nakajima, Associate Professor Fumitoki Watanabe, Assistant Professor Shigeo Koido, Assistant Professor Atsushi Hokari, Assistant Professor Yasuyuki Searashi, Assistant Professor

Research Activities

Alimentary tract

 FOXP3+ CD4+ regulatory T cells accumulate in areas of active inflammation, including granulomas in Crohn's disease, and retain potent regulatory activity ex vivo.
 Visilizumab induces apoptosis of the lamina propria, but not peripheral blood T lymphocytes from patients with ulcerative colitis through activation of caspase-3dependent and caspase-8-dependent pathways.

3. Plasmacytoid dendritic cells (DCs) regulate interleukin10 secretion from regulatory T cells and played a critical role in mucosal repair of colitis.

4. *Helicobacter* infection increased apoptosis in the livers of mice and might play a role in pathogenesis in the liver.

5. DCs engineered to secrete interleukin 12 derived from sarcoma promote the crosspriming of antitumor CD8 T cell responses against hemoglobin-beta.

6. Synergism between OK432-stimulated DCs and heat-treated tumor cells enhances the immunogenicity of DC/tumor fusion cells.

7. The gene mutations encoding inosine triphosphate pyrophosphohydrolase, which influences the metabolism of azathioprine/6-mercaptopurine, are closely related to thiopurine-induced adverse reactions in Japanese.

8. Treatment with cyclophosphamide, doxorubicin, and cisplatin removed both CD16+ and CD16- monocytes in all patients, and the dynamics of the CD16+ monocyte subset differed markedly between responders and nonresponders to treatment with cyclophosphamide, doxorubicin, and cisplatin.

Liver

1. Intrahepatic immunological reaction was studied by model mice of portal vein injection. It was demonstrated of activated CD8+T cells in contact with Kupffer cells and undergoing apoptosis.

2. Intrahepatic expression of the co stimulatory molecule programmed death 1 in autoimmune liver disease: Programmed death 1 was expressed on more than half of the liver-infiltrating T cells within the portal tract.

3. Study of chronic hepatitis B and C virus: Natural killer cells target the hepatitis C virus (HCV) core protein in Cre/loxP-mediated naïve immune responses in HCV

 β 2 division dosage under treatment peg interferon α 2b/ribavirin combination therapy was a predictor response rate of patients with intractable chronic HCV infection. 4. Clinical backgrounds and histological findings in autoimmune hepatitis: Liver

biopsies should be done for both initial diagnosis and the assessment of therapy response in autoimmune hepatitis.

5. The relation of connective tissue growth factor and liver fibrosis: Connective tissue growth factor is a new marker of liver fibrosis with histological findings.

6. Necessity of nutritional evaluation before nutritional support in liver cirrhosis and nonalcoholic liver disease: More than 80% of cases showed excessive food intake. Patients with liver cirrhosis and nonalcoholic liver disease should be evaluated for nutritional imbalance before they receive nutritional support.

7. The expression of survivin during the early stages of hepatocellular carcinoma: In hepatocellular carcinoma tissues, the average survivin expression rate was 62%. The serum level of alanine aminotransferase was correlated with the survivin expression rate in specimens of hepatocellular carcinoma. Some clinical variables may be useful indicators for selecting patients for survivin-inhibiting treatment.

8. The mini-bioartificial liver was cultured with 13 C-glucose, and the amout of 13 CO₂ in exhaust gas reflected the viability of an artificial organ or the damage to it.

9. Development of an implant-type bioartificial liver: A 3-dimensional tissue culture mimicking the liver (liver organoid) was established with an apatite-fiber scaffold in the bioreactor system.

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Department of Internal Medicine Division of Neurology

Kiyoharu Inoue, Professor Hisayoshi Oka, Associate Professor Kazutaka Matsui, Assistant Professor Masahiko Suzuki, Assistant Professor Soichiro Mochio, Professor Akira Kurita, Associate Professor Hironori Sato, Assistant Professor

General Summary

Our research in 2007 was performed in the following areas: 1) dysosmia in Lewy body disease, 2) autonomic dysfunction in Lewy body disease, 3) neuroradiological studies with nuclear medicine, 4) neurophysiological studies on visual cognitive functions in Lewy body disease with dementia, and 5) motor neuron disease.

Research Activities

A simple screening test for dysosmia in Parkinson's disease

Dysosmia in Parkinson's disease (PD) was evaluated with a simple test using an incense stick. The subjects were 83 healthy controls and 60 patients with PD. The percentage of subjects with dysosmia was significantly higher among patients with PD than among the healthy controls. Among patients with PD, dysosmia was associated with a significantly longer duration of disease than was a normal response.

Lewy body in aging human olfactory bulb

We investigated the incidence and extent of Lewy body-related α -synucleinopathy (LBAS) in the olfactory bulb in 320 consecutive patients examined at autopsy. The incidence of LBAS in the aging human olfactory bulb was high. LBAS apparently extends from the periphery to the anterior olfactory nucleus and results in clinical manifestations of Lewy body disease. The result showed that the olfactory bulb is one of the first anatomical sites affected by LBAS, and its functional and morphological evaluation is useful for the diagnosis and clinical evaluation of Lewy body disease.

Cardiovascular autonomic dysfunction in neurological disorders

We studied the cardiovascular autonomic dysfunction in patients with Lewy body disease, such as PD or dementia with Lewy bodies. The autonomic function was evaluated with cardiac ¹²³I-metaiodobenzylguanidine (MIBG) scintigraphy, hemodynamic function testing with the Valsalva maneuver, the orthostatic tolerance test, and spectral analyses of heart rate and blood pressure. Reduced uptake of ¹²³I-MIBG in the heart and cardiovascular autonomic dysfunction was found in patients with early stage PD. We are also evaluated the relationship between reduced uptake of ¹²³I-MIBG in the heart and cardiovascular dysfunction with the Valsalva maneuver, orthostatic tolerance testing, and spectral analyses of heart rate and blood pressure in PD.

Sudomotor dysfunction in PD

We studied the relationship between sudomotor dysfunction and cardiovascular autonomic dysfunction in PD. Sudomotor dysfunction in PD evaluated with an evaporimeter affected the forehead and the upper and lower extremities. The characteristics of sudomotor dysfunction are different from cardiac sympathetic dysfunction in the clinical course of autonomic disturbance in PD.

Neuroradiological studies with nuclear medicine

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

1. Study of cardiac sympathetic dysfunction in1-methyl-4-phenyl-1,2,3,6-tetrahydroxypyridine (MPTP)-induced parkinsonian mice

Our study clearly showed that cardiac ¹²⁵I-MIBG uptake and the total number of binding sites of norepinephrine transporter are rapidly reduced after MPTP administration, followed by a partial recovery of ¹²⁵I-MIBG uptake and an even greater reduction after repeated MPTP administration, coincident with norepinephrine synthesis in mice hearts. 2. Clinical utility of myocardial ¹²³I-MIBG scintigraphy in parkinsonism and dementia

Myocardial ¹²³I-MIBG scintigraphy is useful for differentiating Lewy body disease from other neurodegenerative diseases.

3. ¹²³I-IMP brain single-photon emission computed tomography study in neurodegenerative disease

By reviewing both the decrease image and the increase image as with the Two-Tail View, 3-dimensional stereotactic surface projection may provide more information on the relative distribution of blood flow and metabolism and facilitate the differential diagnosis of parkinsonian disorders using photon emission tomography (PET).

4. Establishment of early differential diagnosis in parkinsonian and demented disorders using PET

PET investigation of presynaptic and postsynaptic nigrostriatal dopaminergic functions may provide clues to understanding the development and advancement mechanism of the disease and will aid in a more reliable early clinical diagnosis and prediction of drug effects.

Neurophysiological studies on visual cognitive functions in Lewy body disease with dementia

Visual information processing functions were assessed with visual event-related potentials in patients with dementia with Lewy bodies, Alzheimer's disease, and PD. The results were presented in part at the 48th annual meeting of the Japanese Society of Neurology. The lead author of these studies was invited as a guest speaker of a symposium at the 37th annual meeting of the Japanese Society of Clinical Neurophysiology. The results in these studies concerning the relationship between visual hallucinations and visual cognitive impairments were presented in the presidential lecture at the 10th annual meeting of the Japanese Pharmaco-EEG Society. *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 and those in the dorsal motor nucleus of the vagus nerve in brainstem slices from young rats. The results suggested that 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 selective degeneration of motor neurons.

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Department of Internal Medicine Division of Kidney and Hypertension

Tatsuo Hosoya, Professor Iwao Ohno, Associate Professor Kimiyoshi Ichida, Assistant Professor Keitaro Yokoyama, Assistant Professor Makoto Ogura, Assistant Professor Masato Ikeda, Assistant Professor Tetsuya Kawamura, Associate Professor Goro Tokudome, Associate Professor Yasunori Utsunomiya, Assistant Professor Yasuhiro Yamamoto, Assistant Professor Kazushige Hanaoka, Assistant Professor Yoichi Miyazaki, Assistant Professor

General Summary

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

Research Activities

Nephrology

1. Glomerulonephritis

The prognosis of patients with IgA nephropathy and metabolic syndrome may be unfavorable because these patients show resistance to renin-angiotensin system (RAS) inhibitors owing to obesity and insulin resistance. Our data demonstrated that plasmalemmal vesicle—associated protein 1 is expressed in the lesions of polar vasculosis and glomerular capillaries in the early stage of diabetic nephropathy, suggesting that the phenotypic change of endothelial cells forming caveolae may occur in the development of diabetic nephropathy. Vascular endothelial growth factor and bone morphogenetic protein 4 can act on the endothelial precursor cells, as a stimulator and an inhibitor, respectively, thereby orchestrating the morphogenesis of glomerular capillary tufts. We successfully induced kidney regeneration without an adenovirus by using an artificial pathogen-free biomaterial as a tool to diffuse glial-cell—derived neurotrophic factor. 2. Dialysis

We found that the changes in bone turnover or in osteoprotegerin itself influenced the response of urinary phosphate excretion via fibroblast growth factor 23 to a high-phosphate diet in osteoprotegerin knockout mice. We also evaluated the role of collagen enzymatic and glycation-induced cross-links as a determinant of bone quality in patients with secondary hyperparathyroidism.

We found that L-type Ca^{2+} channels play a role in the high extracellular Ca^{2+} -activated increase in cytoplasmic Ca^{2+} concentration.

We evaluated the clinical value of the combination of peritoneal dialysis and hemodialysis and found it is a useful way to control body fluids and may allow peritoneal function to be maintained for a long period of time. In transplant glomerulopathy, the glomerular expression of plasmalemmal vesicle — associated protein 1 was found to be positively correlated with the severity of transplant glomerulopathy and proteinuria.

Hypertension

Blockade of T-type Ca channels constitutes a new therapeutic target for protecting against cardiovascular disease in patients with chronic kidney disease. The levels of 24-hour urinary protein excretion, serum creatinine, global glomerulosclerosis, and interstitial damage before pregnancy may be predictors of the risk of hypertension due to superimposed preeclampsia in patients with IgA nephropathy. In male patients with untreated essential hypertension, the serum level of uric acid is an independent marker of systemic arterial stiffness and microalbuminuria.

Uric acid metabolism

The G774A mutation in the SLC22A12 gene encoding urate transporter 1 is common in Japanese patients with renal hypouricemia. This G774A mutation was likely introduced by immigrants from mainland Asia and thereafter expanded in the Japanese population by founder effects or genetic drift or both. Carotid artery intimal-medial thickness in patients with gout or hyperuricemia was suggested to be increased by decreased renal function.

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Department of Internal Medicine Division of Rheumatology

Akio Yamada, 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 effects of neovascularisation inhibitors in animal models of rheumatoid arthritis

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

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

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

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

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

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

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

performed.

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Department of Internal Medicine Division of Cardiology

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

General Summary

We are actively engaged in research activities associated with daily clinical practices, including large-scale clinical trials and multicenter trials. We have been participating in and cooperating with several large-scale clinical trials, notably the Japanese Investigation of Kinetic Evaluation in Hypertensive Event And Remodeling Treatment (JIKEI HEART) study, the Japanese Rhythm Management Trial for Atrial Fibrillation (J-RHYTHM) study, the Assessment of β -Blocker Treatment in Japanese Patients with Chronic Heart Failure (J-CHF) study, the Pitavastatin hEARt faiLure (PEARL) study, and the Combination of OLMesartan and Calcium channel blocker or diuretics in high risk elderly hypertensive patients Study (COLM) study. The JIKEI HEART Study, is a voluntary physician-oriented study initiated by our chairperson. It is the first clinical-outcome cardiology study in Japan to demonstrate that an angiotensin II receptor blocker administration significantly reduces risks. The study results were reported in *The Lancet* (Mochizuki S, et al. Lancet 2007; 369: 1431–9).

Research Activities

Clinical research

1. Ischemic heart disease

Individual clinical research sections have been compiling subject data, such as risk factors and lesional morphology collected through catheterization tests or treatments, into a database that can be used to compare risk factors and prognosis for ischemic heart diseases, such as cardiomyopathy. Furthermore, we have been carefully examining treatments, mainly those using drug-eluting stents, and diagnoses of vasospasm, which is intimately associated with the development of ischemic heart diseases. We have also been participating in nationwide clinical research activities. Coronary artery computed tomographic scanning using dual-source computed tomography started this May, and we are accumulating visualization exposures at a rate of 950 cases per year. Temporal resolution capacity of our current system is greater than that of our former one, enabling us to provide more accurate diagnoses and detailed examinations for cases of arrhythmia or high cardiac pulsation.

2. Heart failure

We have been examining the serum level of brain natriuretic protein as an indicator of heart failure and investigating reference values that can be used in clinical practice. Also, we are now making detailed examinations of the pathological condition of heart failure, both before and after hospital admission, to obtain clinical data for establishing new indicators.

3. Arrhythmia

We used catheter ablation to treat 232 cases of atrial fibrillation this year. In clinical research, we published papers on the following topics: 1) examining the usefulness of pulmonary vein antral isolation using a potential indicator, and 2) suppression of reentry into the signaling pathway by annihilation of reentry into ATP signaling after pulmonary vein antral isolation.

4. Lipid metabolism

With regard to human lipoprotein metabolic research using a stable isotope, we collaborated with Kanazawa University to perform tracer experiments on patients with autosomal recessive hypercholesterolemia, which is an extremely rare condition. Additionally, we examined the effect of ezetimibe, an inhibitor of cholesterol absorption from the small intestine, on lipoprotein metabolism and are now analyzing the data.

Basic research activities

Basic research activities include domestic and international exchanges of graduate students in basic or clinical courses and the publication of the results of their studies. For research into the cause of atrial fibrillation, we are now observing changes in the expression of connexin, which controls intracellular signal transduction, and decreases in cardiac function/pressure load in the Dahl model of hypertension-induced heart failure. We are also evaluating the localization of proteins, such as zona occludens 1, associated with connexin. In cardiomyocyte physiology, we are examining the physiological and pathophysiological regulatory mechanisms of myocardial contraction and relaxation, at both molecular biological and physiological levels. We are also examining a new signal transduction pathway and its effects on alpha-adrenergic receptor stimulation in the L-type Ca channel of rat myocardium, the effects of beta-adrenergic receptor stimulation on the sarcoplasmic reticulum function, and Ca handling in the cardiomyocytes of mice with dilated cardiomyopathy induced by troponin T mutation. In myocardial metabolism, we have been evaluating the relationship between ischemic reperfusion injury and intracellular ionic handling using isolated perfused hearts from a mouse model of type 2 diabetes.

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Department of Internal Medicine Division of Diabetes, Metabolism and Endocrinology

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

Genetic epidemiology of diabetes mellitus

A loss or decrease in the function of glutathione peroxidase (GPX) 1, an antioxidant enzyme, is crucial for the development of coronary atherosclerosis. This year, we have found that the single nucleotide polymorphism in complementary DNA (Pro197Leu substitution) of GPX-1 is genetically associated with coronary artery calcification detected with multislice computed tomography and have published our findings in a leading journal. We are planning a prospective study that enables us to perform a new type of individualized medicine.

Gene and cell-based therapy for diabetes mellitus

To restore the pancreatic beta cell mass and restore glucose metabolism in diabetes mellitus, we have transferred the CDK4 gene, a gene for a regulatory factor of the cell cycle, to animals with diabetes. We found that the reduced beta cell mass was increased and that blood glucose levels were decreased. We are preparing our findings for publication in a leading journal.

Diabetic vascular complications

Research has focused on the pathogenesis and treatment of diabetic vascular complications. Clinical studies have examined dietary therapy for type 2 diabetes. Experimental studies using vascular smooth muscle cells, mesangial cells, and retinal pericytes have investigated the role of the Rho/Rho-kinase-mediated signaling pathway in the pathogenesis of diabetic vascular complications and have provided evidence that these molecules are potential pharmacological targets in the treatment of diabetic vascular injury.

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 new oral hypoglycemic agents on insulin resistance were investigated.

Endocrinology

The potential role of inflammatory cytokines on the dynamics of the urocortincorticotropin-releasing hormone receptor system in HL-1 cardiomyocytes was evaluated. The involvement of corticotropin-releasing hormone receptor signaling against vascular inflammatory stress was studied in human aortic endothelial cells.

The molecular pathogenesis of impaired negative feedback mechanism by glucocorticoids in the growth and development of pituitary macroadenoma causing subclinical Cushing's disease was investigated.

Immunopathological analysis was performed of pituitary tissue obtained at autopsy in a case of acquired combined pituitary hormone deficiency.

Clinical features of 5 patients with subclinical Cushing's disease were characterized.

Dietary therapy

The effects of a high monounsaturated fatty acid enteral formula versus a highcarbohydrate enteral formula on postprandial plasma glucose concentrations and insulin response was examined in patients with type 2 diabetes mellitus and in healthy volunteers. A high-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 healthy subjects.

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Department of Internal Medicine Division of Clinical Oncology/Hematology

Keisuke Aiba, Professor Fumi Mizorogi, Professor Daisuke Inoue, Associate Professor Toshikazu Sakuyama, Assistant Professor Osamu Asai, Assistant Professor Shuichi Masuoka, Assistant Professor Shingo Yano, Assistant Professor Tadashi Kobayashi, Professor Noriko Usui, Associate Professor Toshio Katayama, Assistant Professor Takaki Shimada, Assistant Professor Nobuaki Dobashi, Assistant Professor Yoshikazu Nishiwaki, Assistant Professor

General Summary

The immediate goals of our clinical and basic research are to investigate basic and clinical aspects of malignant diseases and to try to improve outcomes for patients with hematological malignancies and solid tumors, leading to the ultimate goal of improving the natural history of malignant diseases. We have also been performing several clinical trials and basic research studies throughout 2007.

Leukemias

Many patients with previously untreated hematological disorder have been referred to our department. The disorders include acute myeloid leukemia (AML), 16 cases; acute promyelocytic leukemia (APL), 2 cases; acute lymphoblastic leukemia (ALL), 7 cases, including 3 cases of Philadelphia chromosome - positive ALL; chronic myeloid leukemia (CML), 5 cases; and myelodysplastic syndrome (MDS), 10 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 2007 were as follows: AML/MDS-HR CS-7 study (newly diagnosed AML, refractory anemia with excess blasts II, all case registration: cohort study), AML-201 VLA4 study (prognosis evaluation study), AML-206 DNR assigned group (relapse and refractory AML: phase I), APL-204 (phase III), APL-205R (relapsed and refractory APL: phase II), and ALL-202 (phase III). We also performed several cooperative group studies and pilot studies: Aged Double-7 (newly diagnosed aged AML: phase II), V EGA (MDS: phase II), nilotinib (refractory CML: phase I/II), and dasatinib study (refractory CML: phase I/II).

Lymphomas

In 2007 we registered patients with newly diagnosed non-Hodgkin's lymphoma (NHL), 65 cases, or Hodgkin's lymphoma (HL), 5 cases. We have performed clinical trials as a member of the Lymphoma Study Group of the Japan Clinical Oncology Group (JCOG-LSG). The studies JCOG0211-DI (newly diagnosed natural killer/T lymphoma: phase I/II) and JCOG0203-MF (newly diagnosed follicular lymphoma: phase III) were pivotal protocol studies in 2007. Other cooperative studies examined

biweekly rituximab, etoposide, prednisone, vincristine, hydroxydaunorubicin (R-EPOCH; relapsed and refractory B cell lymphoma: phase II), pirarubicin, cyclophophamide, vincristine, and prednisolone (THP-COP; newly diagnosed T-cell lymphoma: phase II), and enzastaurin (NHL: phase III, double blind). Enzastaurin is a novel drug targeting protein kinase $C\beta$ that has been extensively studied throughout the world, including the United States, the European Union, and Japan.

Myeloma

We completed our original pilot study evaluating a combination of thalidomide and dexamethasone in 2007. A novel agent, the proteasome inhibitor bortezomib, became available in 2007, and we have used it to treat patients who have refractory myeloma.

Hematopoietic stem cell transplantation

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

Solid tumors

Several of our studies are in progress throughout our university hospital with related divisions or departments, seeking improved therapeutic outcomes. Fluorouracil (5-FU), 500 mg/m^2 ; epirubicin, 100 mg/m^2 ; and cyclophosphamide, 500 mg/m^2 (FEC100) with or without taxotere therapy is an adjuvant therapy for patients with breast cancer treated with curative surgery. FEC100 followed by taxotere is a preoperative combination chemotherapy for patients with locally advanced breast cancer. AT - followed by taxotere and herceptin is a first-line chemotherapy for patients with advanced metastatic breast cancer. The standard treatment for operable, locally advanced esophageal cancer has been altered, resulting in the use of chemoradiation therapy rather than surgical resection. We, therefore, have been investigating a combined modality therapy of radiation and chemotherapy with low-dose cisplatin and 24 hours' continuous infusion of 5-FU for such patients since 2002. The results will be reported next year. For patients with advanced gastric cancer, a combination chemotherapy of S-1 and cisplatin has been performed. Our first-line chemotherapies for patients with advanced colorectal cancer are folinic acid, 5-FU, and irinotecan (FOL-FIRI) and folinic acid, fluorouracil, and oxaliplatin (FOLFOX).

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 improved the control of cancer pain.

In our new division, we aim to attain individual goals by sharing our thoughts and to contribute to the further growth of palliative care at The Jikei University School of Medicine.

Basic research

One of our important activities is translational research on hematological malignancies and solid cancers. The structural differences between M protein produced by myeloma cells and that from monoclonal gammopathy of undetermined significance have been examined, and the function of ATP-binding cassette transporters in cancer chemotherapy has also been studied in collaboration with the Kyoritsu University of Pharmacy (Keio University Department of Pharmacy).

Publications

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Department of Internal Medicine Division of Respiratory Diseases

Kazuyoshi Kuwano, Professor Akira Kojima, Associate Professor Hiroshi Takeda, Assitant Professor Hisakazu Tai, Associate Professor Heiichi Yano, Assistant Professor Katsutoshi Nakayama, Assitant Professor

General Summary

We have started 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 develop novel treatments.

Research Activities

COPD

Clinical research concerning the incidence of COPD in patients with diabetes mellitus was started.

Pulmonary infection: The investigation of biomarkers for infectious lung diseases was started.

Lung cancer

Clinical research about the effects of nitroglycerin on chemotherapy in non-small cell lung cancer was started. This study is a multicenter trial in Japan.

Pulmonary fibrosis: Basic research concerning the mechanism of transforming growth factor beta activation by integrin and its role in epithelial and mesenchymal cell interactions was started.

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Department of Internal Medicine Division of General Medicine

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

Research Activities

Division of General Medicine, The Jikei University Hospital

Little is known about the relationship between anxiety and health-related quality of life (QOL) in a primary care setting. The objective of this study was to determine whether the degree of anxiety affects physical complaints and the QOL physical status scale. The State Trait Anxiety Inventory (STAI) and the 36-Item Short Form Health Survey (SF-36) were used to evaluate the degree of anxiety and health-related QOL scores, respectively. To date, 46 patients (26 men and 20 women) with a mean age of 41.2 ± 12.8 years have participated in the study. Of the 25 physical complaints, those correlated with anxiety were general fatigue, vertigo, and dizziness. The physical component summary of the SF-36 showed a statistically significant correlation with anxiety. The physical component summary of the SF-36 showed a statistically significant correlation with anxiety.

Division of General Medicine, The Jikei University Aoto Hospital

Experimental and clinical investigations were performed for cardiovascular abnormalities in hypertension, diabetes, and cardiomyopathy.

1. Cardiovascular damage increases if patients with hypertension also have diabetes. The effects of combination therapy with antihypertensive drugs were investigated in patients with hypertension and associated diabetes mellitus. The effects of angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, calcium antagonists, and beta adrenergic blockers with or without intrinsic sympathomimetic action were examined.

2. An experiment was performed to investigate alterations in myocardial subcellular organelles in cardiomyopathic J2N-k hamsters. A molecular biological examination was also performed for laminin, which is a component of the extracellular matrix.

3. The effects of *shinrin-yoku* (forest-air bathing and walking) on sympathetic nervous activity is being examined in healthy elderly persons.

Division of General Medicine, 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 nutritional state, administered drugs, and biochemi-

cal markers.

2. Study of fever of unknown origin

We attempted to clarify the cause of fevers 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. Since 2000 we have treated 500 patients with fever of unknown origin. We found that viral infection can be distinguished from bacterial infection on the basis of the results of these measurements.

Division of General Medicine, Jikei University Kashiwa Hospital

Our research works consist of three parts: the first part is to investigate the role of the general medicine on the environmental health achievement in regional areas, especially in Kashiwa City; the second part is to evaluate lipid abnormalities that result in premature atherosclerosis and to develop treatments for atherosclerotic disorders; and the third part is to develop educational tasks for teaching medical students and junior residents.

1. Investigation of the role of general medicine on environmental health achievement We developed a new regional nutritional education system and reported practical procedures at the 29th annual meeting of the Japanese Society of Clinical Nutrition in Kyoto.

2. Studies of lipid metabolism and atherosclerosis

1) Using a newly developed method of high-performance liquid chromatography, we demonstrated that oxidized low-density lipoprotein elevates release of serotonin from platelets (Am J Haematol 2007).

2) Diacylglycerol ingestion was found to increase plasma levels of serotonin, which stimulates thermogenesis, and suggests a new mechanism for treating obesity with diacylglycerol (J Clin Lipidol 2007).

3) Diacylglycerol ingestion was found to suppress postprandial hyperlipidemia in a subject with genetic hyperchylomicronemia (QJM 2007).

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

5) Supervised regular exercise training for 4 months was found to increase adiponectin levels, but exercise for 2 months had a less significant effect.

6) Evaluation of serum levels of very low density lipoprotein cholesterol was found to be a useful measure for monitoring lipoprotein amelioration achieved by exercise.

7) Subanalysis of the Japanese Investigation of Kinetic Evaluation in Hypertensive Event And Remodeling Treatment study showed that antihypertensive therapy with an angiotensin II receptor blocker prevents cardiovascular events in Japanese women.

3. Medical Education

1) A new practical algorithm for evidence-based medicine was developed for the clinical setting.

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

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Department of Psychiatry

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

General Summary

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

Research Activities

Psychopathology, psychotherapy, and child psychiatry study group

Two main studies were performed in the fields of psychopathology and psychotherapy. The first study concerned the effectiveness of short-term and moderately structuralized treatment for inpatients with borderline personality disorder. The second study investigated, from the viewpoint of mental health in the office, the background factors of patients who were absent from work because of mental disorders. A text on dialectical behavioral therapy by Marsha Linehan was translated into Japanese and was published.

Morita therapy group

A study established the effectiveness of Morita therapy for social anxiety disorder. In addition, outpatient Morita therapy was standardized, and guidelines for it are being established. Studies involved: 1) character profiles of patients with chronic depression, 2) the subjective experience of patients who showed improvement through inpatient Morita therapy, 3) the relationship between panic disorder and generalized anxiety disorder from the aspect of patient's character and comorbidity, and 4) psychopathological research on "fall-in reaction" occurring in the process of mood or anxiety disorders. In addition to the above studies, research on the subtypes of the obsessive compulsive disorder was started.

Psychopharmacology group

In basic research, the mechanism of the central action of a new generation of psychotropic drugs was studied with microdialysis or radioimmunoassay, and brain mechanisms underlying the processes of learning and memory in drug dependence were studied in rats. Clinical research included a study of the clinical characteristics of craving for drugs of abuse regarding the difficulty in abstaining from them, a positron emission computed tomography study of brain receptors in patients with mental disorders, molecular biological studies of mental disorders in corroboration with

Department of Virology, and rational pharmacotherapy with second-generation antipsychotic agents.

Psychophysiology group

Studies examined differences in the side effects of benzodiazepine and nonbenzodiazepine hypnotics in regard to their modes of action on benzodiazepine receptors, the effects of selective serotonin reuptake inhibitors on obstructive sleep apnea-hypopnea syndrome, the effectiveness of continuous positive air-way pressure on obstructive sleep apnea-hypopnea syndrome, the effects of cognitive behavioral therapy and Morita therapy on psychophysiological insomnia, and the effects of *kanyokusan* and quetiapine on the cyclic alternating pattern as an index of sleep structure. In addition, clinical studies examined refractory insomnia and sleep disturbance in patients with functional gastrointestinal disorders.

Psychogeriatric group

The epidemiological studies in Niigata Prefecture Itoigawa City that were started in 1998 have been continued. These studies investigated the current use and costs of nursing care insurance and the predictors of the risk of mortality in the elderly. We have planned another study of mental disorders in patients with breast cancer, with a focus on the relationship with demographic factors and physical state, in cooperation with the general hospital psychiatry research group and the Department of Surgery. In addition, we started to open a clinic specializing in dementia.

General hospital psychiatry

We have continued treatment based on cognitive-behavioral therapy to prevent recurrences of depression. To improve this therapy, we have tried to use a computer system for more effective presentations and have introduced a new system to more accurately evaluate sleep. Furthermore, we investigated new indications for this therapy, such as insomnia and atypical depression. Collaborative studies were performed with the National Cancer Center Hospital East to investigate psychological distress in the family members of cancer patients. Furthermore, supportive mental care was started for cancer patients, their families, and medical staff.

Clinical electroencephalography group

We have continued a study reconsidering the clinical characteristics of classical electroencephalograms (EEGs), and, through an investigation of the 6-Hz spike and wave complex, we have clarified that the affinity for histamine H1 receptors of secondgeneration antipsychotic drugs is related to abnormal EEGs or epileptic seizures or both. Additionally, an investigation of cases that are difficult to diagnose with EEG is under way in collaboration with research groups of the Departments of Psychophysiology and Neurosurgery.

Clinical psychology group

We have continued to discuss and study psychotherapeutic processes and the treatment

techniques of psychoanalytic psychotherapy, Morita therapy. and counseling. Furthermore, we have examined the characteristics of developmental disorders and various types of personality disorders through psychological assessments. We also studied clinical issues and educated trainees. Clinical psychologist Takei Itoh was invited to lecture at a clinical conference, where the evaluation of developmental disorders was discussed.

Publications

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Department of Pediatrics

Yoshikatsu Eto, Professor Fumiyuki Ito, Professor Nobuo Usui, Professor Tohya Ohashi, Professor Toshio Katsunuma, Associate Professor Ichiro Miyata, Associate Professor Hiroaki Wakasugi, Assistant Professor Yoko Kato, Assistant Professor Yoshihiro Hayashi, Assistant Professor Hiroshi Kobayashi, Assistant Professor Masakatsu Kubo, Professor Yasutaka Hoshi, Professor Hiroyuki Ida, Professor Hisashi Tamaki, Associate Professor Yasuyuki Wada, Associate Professor Mitsuyoshi Urashima, Associate Professor Masako Fujiwara, Assistant Professor Yoshihiro Saito, Assistant Professor Hiroshi Tachimoto, Assistant Professor Masaharu Akiyama, Assistant Professor

General Summary

We have 8 subspeciality research groups: 1) the Medical Genetics, Congenital Metabolic Diseases, Endocrinology, Gastroenterology and Hepatology group, 2) the Allergy and Immunology group, 3) the Neurology group, 4) the Hematology and Oncology group, 5) the Cardiology group, 6) the Infectious Diseases group, 7) the Neonatology group, and 8) the Nephrology group. The ultimate aim of these subspecialty groups is to supply practical benefits to patients and their families through basic and clinical research. Realizing this aim requires cooperation and a high motivation for research.

Research Activities

Medical genetics, congenital metabolic diseases, endocrinology, gastroenterology and hepatology

We focused on research concerning medical genetics, congenital metabolic diseases, endocrinology, gastroenterology, and hepatology. In the field of medical genetics and congenital metabolic diseases, we analyzed the clinical manifestations and natural history of Japanese heterozygous females with Fabry disease. Furthermore, we studied the effect of antibody formation on the reduction in urinary levels of globotriaosylcer-amide during agalsidase beta therapy in patients with Fabry disease. In the field of endocrinology, we performed molecular analysis of autosomal dominant hypoparathyroidism in a neonate and studied intrauterine therapy for fetal goiterous hypothyroidism during late gestation. In the field of gastroenterology and hepatology, we performed a retrospective multicenter study to examine the association between gastric atrophy and *Helicobacter pylori* infection in Japanese children.

Allergy and immunology

We have been measuring several types of marker in exhaled breath condensate from young children with asthma. Next, we measured the levels of exhaled nitric oxide in young children with asthma and compared them with levels in control subjects without asthma (4.8 ± 0.7 ; age range, 2 to 6 years). We found that levels of exhaled nitric oxide were negatively correlated with symptom-free periods (r=-0.239, p=0.044).

The mechanism of asthma exacerbation induced by upper respiratory infection with rhinovirus has also been investigated.

We developed a questionnaire for caregivers of children with atopic dermatitis (Quality of Life of Caregivers of Children with Atopic Dermatitis) which has been submitted for publication.

Neurology

We focused on 2 research activities concerning human herpes virus (HHV) 6 encephalopathy and West syndrome last year. First, we investigated HHV-6 encephalopathy, to classify HHV-6 encephalopathy based on the findings of magnetic resonance imaging and single-photon emission computed tomography. HHV-6 encephalopathy can be classified into 4 types: 1) the frontal predominant type, 2) the hemispheric type, 3) the acute necrotizing encephalopathy type, and 4) the diffuse type. The hemispheric type predominantly affected the occipital lobes. The diffuse type showed decreased blood flow in all areas of the cerebrum and had a frontal predominance similar to that of the frontal predominant type. All patients with the hemispheric type had frequent clusters of hemiconvulsions both in the initial febrile period and in the defervescence period, which were followed by transient hemiplegia. Patients with the frontal predominant type or the diffuse type had generalized convulsions, including secondary generalized seizures, in clusters in the defervescence period. This imaging-based classification of HHV-6 encephalopathy suggests that each type of encephalopathy has characteristic manifestations. Pathophysiologic changes, such as direct viral invasion, vascular changes, cytokine storm, and secondary immunological response, seem to be reflected in the type of HHV-6 encephalitis, and each type may be associated with characteristic manifestations.

Next we describe our study of West syndrome. We studied factors affecting developmental outcomes in patients with cryptogenic West syndrome. Medical records of 32 patients with West syndrome were reviewed for clinical features: treatment lag, electroencephalography findings, and seizure evolution. Those features were compared between a good outcome group and a poor outcome group. The duration from onset to any treatment was longer in the poor outcome group than in the good outcome group. Evolution of electroencephalography findings showed that paroxysmal discharges reappeared in frontal regions more frequently in the poor outcome group than in the good outcome group. Frequency of other type of seizure except spasms was higher in the poor outcome group than that in the good outcome group. Focal epilepsy developed more frequently in the poor outcome group than in the good outcome group. In conclusion, shorter treatment lag is associated with a favorable outcome in cryptogenic West syndrome. Reappearance of paroxysmal discharges in the frontal regions and the evolution to other types of seizure may be associated with undetectable lesions in the frontal region.

Hematology and oncology

We demonstrated the molecular mechanism of the antitumor activity of the Gquadruplex-interacting agent 5,10,15,20-tetrakis (*N*-methyl-4-pyridyl) porphyrin (TMPyP4) through G-quadruplex stabilization in guanine-rich DNA sequences in K562 leukemic cells. Moreover, we reported that the effects of an inhibitor of epidermal growth factor receptor were dependent on the EGFR mutation pattern. We investigated the expression of E-cadherin and N-cadherin in paraffin-embedded sequential surgical specimens and autopsy specimens from a 4-year-old girl with recurrent ependymoma and subsequent cerebrospinal fluid dissemination. Expression of E-cadherin was low in all surgical specimens and autopsy specimens, whereas expression of N-cadherin was high level in all surgical specimens but was decreased in autopsy specimens. These results suggest that the expression of N-cadherin is a marker for cerebrospinal fluid dissemination in ependymoma.

Cardiology

Our cardiologic studies were as follows.

- 1. Prenatal diagnosis of congenital heart disease
- 2. Diagnosis, treatment, and long-term postoperative follow-up of congenital heart disease
- 3. Basic assessment of right ventricular failure
- 4. Multidetector-row computed tomography in congenital heart disease
- 5. Evaluation of respiratory circulation dynamics with expired gas analysis for children with heart disease
- 6. Strategy for the treatment of acute-phase Kawasaki disease
- 7. Evaluation of respiratory function in congenital heart disease
- 8. Treatment of arrhythmia detected with cardiac screening in school-aged children
- 9. Epidemiology of Kawasaki disease
- 10. Magnesium dynamics in pediatric cardiology
- 11. Magnesium therapy for arrhythmia in childhood
- 12. Molecular biology in congenital heart disease
- 13. Dynamics of nitric oxide in children with congenital heart disease
- 14. Secretion kinetics of atrial and brain natriuretic peptides in children with congenital heart disease
- 15. Catheter intervention for congenital heart disease
- 16. Thyroid function in congenital heart disease
- 17. Ventricular function in patients who have undergone the Fontan procedure
- 18. Assessment of cardiac function in metabolic disease

We perform research after finishing our daily practice. We presented many findings at annual meetings.

Infectious diseases

Our research focuses on primary immunodeficiency, infectious diseases, and collagen diseases in children. We have been studying new methods of diagnosis and treatment based on our clinical experiences. Our research studies were as follows.

- 1. The diagnosis and gene therapy of chronic granulomatous disease
- 2. Surveillance of respiratory infection
- 3. Efficacy and safety of vaccines

4. Disease activities and prognosis of juvenile idiopathic arthritis and systemic lupus erythematous

5. Effect of molecular intervention against refractory collagen diseases

Neonatalogy

We studied risk factors for patent ductus arteriosus (PDA) with and without spontaneous closure of the ductus arteriosus in very low birth weight infants. Gestational age and surfactant administration were identified as risk factors for PDA. Birth weight, 1-minute Apgar score, mechanical ventilation, respiratory distress syndrome, and red blood cells were also identified as significant risk factors for PDA.

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Department of Dermatology

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

General Summary

We have organized special clinics for selected skin diseases, including viral diseases, neurofibromatosis type 1 (NF1), atopic dermatitis, psoriasis, collagen vascular diseases, and skin cancers. Integrating concentrated clinical efforts and related basic research should provide a significant contribution to excellent clinical practice.

Research Activities

Psoriasis

Various systemic therapies, including oral cyclosorin, methotrexate and etretinate in addition to topical vitamin D3 and steroids, have been used depending on the disease severity and the degree to which quality of life (QOL) is impaired. Also phototherapy, including psoralen ultraviolet (UV) A and narrow-band UVB, is effective and is being performed in a newly organized skin-care clinic. We have evaluated patients' QOL, reflecting social background, and have developed a Japanese version of the Psoriasis Disability Index. In a special psoriasis clinic, we select patient-based treatments to satisfy patients' demand. Clinical trials of new biologic agents, including infliximab, adalimumab and ustekinumab, have been performed. We have organized twice-yearly meetings in the auditorium of our university with patients in the Tokyo area 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 be involved in the exacerbation of atopic dermatitis. Therefore, we are trying to treat patients on the basis of evidence-based medicine and of QOL issues. We are trying to obtain a complete medical history and assess how QOL is impaired for each patient. To support such an approach, we have organized twice weekly skin-care lessons in the Skin Care Clinic and a monthly atopic dermatitis forum, which includes lectures and group meetings. In basic clinical research, levels of substance P and interleukin 31 related to pruritus in atopic dermatitis were evaluated according to the disease severity. A clinical trial of a topical nuclear factor- κ B decoy has been performed.

Malignant skin tumors

We have been studying clinical courses and postoperative outcomes of patients with

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

In particular, sentinel lymph-node biopsy is performed for patients with stage II or III melanoma. We are participating in cooperative clinical research on maintenance therapy using local interferon- β injection.

Neurofibromatosis

Because our clinic has the largest number of registered patients in Japan and treats many patients from all over Japan who bring letters of introduction, we concentrate on long-term follow-up and improvement of QOL through accurate diagnosis and resection of neurofibromas. Because the lifetime risk of malignant peripheral nerve sheath tumor (MPNSTs) in patients with NF1 is estimated to be 10% and because surgical removal is the most effective treatment, MPNST must be diagnosed as early as possible. With ¹⁸fluorodeoxyglucose positron emission tomography (FDG-PET), which is safe and highly sensitive, we could detect MPNST at an early stage in 3 patients with deep, hard tumors, demonstrating that FDG-PET is more useful for detecting MPNST than is magnetic resonance imaging.

Herpes virus infection

1. Herpes simplex virus

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

A survey of QOL in patients with recurrent genital herpes and drug sensitivities derived from HSV from recurrent genital herpes is now being performed.

2. Herpes zoster and postherpetic neuralgia

Initial treatments for herpes zoster and postherpetic neuralgia (PHN) are performed in this clinic. PHN is a major sequela of VZV infection and decreases QOL. To control PHN, we are prescribing selective serotonin reuptake inhibitors and investigating the efficacy of other new drugs.

Human papillomavirus infection

In addition to standard cryotherapies, topical vitamin D3 and salicylic acid have been used in the treatment of viral warts. In addition, contact immnunotherapy with squaric acid dibutyl ester and CO_2 laser evaporation has been used for recalcitrant viral warts. Human papillomavirus typing with the polymerase chain reaction method has been

performed regularly in cases of condyloma and rare viral warts. Five-percent imiquimod cream is now available for the treatment of condyloma.

Collagen vascular diseases

Detailed, regularly scheduled follow-up is performed for patients with systemic lupus erythematosus, systemic sclerosis, dermatomyositis, localized scleroderma, Behçet's disease, autoimmune vascular diseases, and photosensitivity diseases.

Contact dermatitis/drug eruption

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

Laser

This year, 1170 patients were treated in the Dermatology Laser Unit. The Q-switched ruby laser is useful for treating nevus Ota because of its selective photothermolysis. Superficial pigmented lesions, such as senile pigment freckles are usually successfully treated with one treatment. Nevus spilus is difficult to treat with the Q-switched ruby laser because it often recurs 1 to 2 months after treatment. The efficacy of a pulsed dye laser for treating hemangiomas and teleangiectasia depends on the clinical type, location, patient age, and other factors. The pulsed dye laser was effective for hemangioma simplex on the face or neck of young adults. The size and intensity of the strawberry mark can be reduced if treatment is started before the age of 6 months. The recently introduced V-beam laser is expected to be effective for recalcitrant 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 lesions that have been increasing in elderly persons.

Skin care clinic

Narrow-band UVB irradiation is performed for patients with psoriasis, atopic dermatitis, prurigo nodularis, vitiligo and cutaneous T-cell lymphomas. The 308-nm excimer lamp is also used. Other special clinics, including those for skin-care lessons, therapy make-up, acne care, mental care, and *kampo* medicine, are open for patients on demand.

Self-assessment

Psoriasis: To improve QOL and improve treatment compliance, we have selected therapies on the basis of the risk/benefit ratios. Phototherapy using narrow-band UVB is actively introduced. Clinical trials by new biologic agents have been performed.

Neurofibromatosis: Many patients with NF1 are still being referred to our special clinic. We are now performing inheritance consultation for pediatric patients. Surgical removal of different types of neurofibromas is performed in inpatient and outpatient clinics to enhance QOL.

Herpes virus infection: We have developed the LAMP method for rapid and sensitive diagnosis. Selective serotonin reuptake inhibitors have proven to be effective for the treatment of PHN.

HPV infections: We have used new treatments, including topical vitamin D3, as well as standard surgical treatments to treat viral warts. HPV typing is also regularly performed.

Contact dermatitis: Causative chemicals, environmental allergens, drugs, and foods in patients with contact dermatitis, drug eruption 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 taken into account. To help patients understand their disease, we have been organizing monthly atopic dermatitis forums, which include lectures and group meetings. Basic research is focused on pruritogens, such as substance P and interleukin 31.

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

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

Collagen vascular diseases: Detailed, regularly scheduled follow-up is performed in cooperation with other departments.

On the basis of the results of many clinical and basic research studies, appropriate treatments can be selected for diverse aspects of skin diseases in our department.

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Department of Radiology

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

General Summary

The division of diagnostic imaging

1. Diffusion-weighted magnetic resonance imaging of neoplastic lesions Diffusion-weighted imaging (DWI) is a new magnetic resonance (MR) technique that evaluates the diffusion capacity of water molecules. With recent advances, the usefulness of DWI for detecting neoplastic conditions has been explored. We are now evaluating the tumors described below to clarify the usefulness and applicability of DWI.

Detectability of hepatocellular carcinoma with DWI in comparison with findings of dynamic computed tomography (CT) and CT portography/CT hepatic arteriography
Usefulness in evaluation of primary lesions, nodal metastasis, and metastasis to the liver of colorectal cancers in comparison with examination of surgical specimens

• Usefulness in evaluation of disease extent of breast cancers in comparison with examination of surgical specimens

• Usefulness in evaluation of prostate cancer: Setting of an optimal b factor for the detection of prostate cancer in high-risk patients and the appropriate level of prostate-specific antigen for performing DWI

2. Multidetector-row CT as a preoperative evaluation for partial hepatectomy

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

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

A study was performed to evaluate the usefulness of nonenhanced 3D heavily T2weighted images obtained with 2D prospective acquisition and correction in the visualization of the lymphatic system of the trunk.

4. Evaluation of the enhancement of the deep venous system of the lower extremities with different total doses and concentrations of iodine in contrast media

A study was performed to assess the effects of different total iodine doses and concentrations on the enhancement of the deep veins of the lower extremities with spiral CT venography.

5. Sonazoid is a second-generation sonographic contrast material. We investigated the MI value of the ultrasound beam, several postprocessing image reconstructive methods, and the application of software to obtain the most useful images with various

types of sonographic equipment, using the contrast material.

6. In a collaborative study with the department of digestive internal medicine, we investigated the timing of liver tumor imaging with contrast materials.

7. To obtain images of sentinel lymph nodes, we performed harmonic sonographic imaging of the pig after subcutaneous injection of contrast material.

8. To clarify the method for obtaining precise 3D images of the vascular structure of superficial soft-tissue tumors, we examined the breast and thyroid tumors using 3D/4D transducer with B flow imaging and developed the most useful 3D imaging method.

9. In collaboration with the department of clinical examination, Tokyo Rika University, and the Aloka Co., Ltd., we developed microbubble contrast materials with antibodies to perform molecular imaging and establish a new drug delivery system.

10. We evaluated the usefulness of several elastic imaging methods to accurately diagnose superficial soft-tissue tumors.

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

12. Fatty acid metabolism of the rat heart in renal failure

Cardiovascular disease is the best predictor of mortality in patients with chronic renal disease. The imaging of fatty acid analogues is useful for diagnosing changes in myocardial metabolism due to uremia and dialysis. We investigated the metabolism of iodine-125-labeled 15-(p-iodophenyl)-3-(R,S)-methyl pentadecanoic acid (BMIPP) the hearts of rats with renal failure. The fatty acid metabolism in the hearts of rats with renal failure between the severity and prognosis of cardiovascular events complicated by renal failure.

13. Palliative pain relief treatment with strontium-89 for multiple bone metastases The osteophilic radiopharmaceutical Sr-89 has been used as a palliative treatment for patients with bone pain caused by bone metastases. Sr-89 is a suitable isotope because it is a pure beta emitter. We obtained Sr-89 images with bremsstrahlung in patients 1 week after injection. Abnormal uptake was seen in all and was consistent with the results of Tc-99m hydroxymethylenediphosphate imaging. Imaging with Sr-89 has not been previously reported. The number of cases of bone metastasis is increasing; therefore, we expect this drug to be useful. We are attempting to determine the effectiveness of Sr-89 and combined therapy with zoledronic acid or other anticancer agents.

The division of radiation therapy

1. Factors affecting treatment outcomes for patients with T2N0 glottis carcinoma

treated with definitive radiotherapy

Purpose: To determine the prognostic factors affecting local outcomes for patients with T2N0 glottis carcinoma treated with definitive radiotherapy.

Methods: The subjects were 48 patients with T2N0 squamous cell carcinoma treated with definitive radiotherapy from 1992 through 2005. The cumulative rates of overall survival, cause-specific survival, local control, and larynx preservation were calculated with the Kaplan-Meier method, and the prognostic significance of patient's age, number of subsites involved, impaired vocal cord mobility, anterior commissure involvement, total dose, and overall treatment time were analyzed with the log-rank test in univariate analysis, and with Cox regression in multivariate analysis. The length of follow-up ranged from 13 to 141 months (median, 62 months).

Results: Five-year survival rates were: overall, 95.3%; and cause-specific, 97.9%; and 5-year rates were local control, 61.4%; and larynx-preservation, 76.4%. Multivariate analyses of the 5 variables showed that overall treatment time significantly influenced the probability of local control and that impaired mobility and overall treatment time affected the probability of larynx preservation.

Conclusion: Our study showed that longer overall treatment time significantly worsenes the rates of local control and larynx preservation for patients with T2N0 glottis carcinoma treated with definitive radiotherapy. Therefore, we advocate a shorter treatment course.

2. Multicenter randomized trial for high dose rate prostate brachytherapy combined with 3D conformal radiotherapy and long-term adjuvant hormonal therapy for high-risk prostate cancer

For the past 2 years we have treated high-risk prostate cancer with such multimodality treatments as high dose rate (HDR) brachytherapy, 3D conformal radiotherapy (CRT), and long-term adjuvant hormonal therapy. In these primarily experiences, we have gradually increased the prescribed doses for both HDR brachytherapy and 3D-CRT to enhance the local control of high-risk prostate cancer. Although systemic therapy is needed for high-risk patients, the optimal duration of the adjuvant hormonal therapy combined with the HDR brachytherapy and external beam treatment remains unclear. Therefore, we have planned a multicenter prospective randomized trial for high-risk prostate cancer to determine the optimal duration of adjuvant hormonal therapy with HDR brachytherapy and 3D-CRT. The duration of adjuvant hormonal therapy will be randomized to two arms as 1 year or 2 years. The prescribed doses to the planning target volume of the prostate are 22 Gy (11 Gy \times 2 fractions) and 40 Gy (2.5 Gy \times 16 fractions), respectively.

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Department of Surgery Division of Digestive Surgery

Katsuhiko Yanaga, Professor Kazuhiko Yoshida, Professor Nobuyoshi Hanyu, Associate Professor Kazuo Matai, Assistant Professor Norio Mitsumori, Assistant Professor Yuji Ishii, Assistant Professor Shuzo Kono, Assistant Professor Tomoyoshi Okamoto, Assistant Professor Hiroshi Nimura, Assistant Professor Hidejirou Kawahara, Assistant Professor Susumu Kobayashi, Professor Hideyuki Kashiwagi, Associate Professor Tetsuji Fujita, Associate Professor Akira Yanagisawa, Assistant Professor Yoshiyuki Furukawa, Assistant Professor Kouji Nakada, Assistant Professor Yuichi Ishida, Assistant Professor Takeyuki Misawa, Assistant Professor Yutaka Suzuki, Assistant Professor Noburo Omura, Assistant Professor

General Summary

The number of operations performed in the Division of Digestive Surgery has been increasing each year. Technically complex operations, such as esophagectomy, laparoscopic total gastrectomy, major hepatectomy, and pancreaticoduodenectomy, have better outcomes when performed at high-volume medical centers than at low-volume centers. However, the quality of surgical care is not only related to volume but also to specific training and interest in particular pathologic conditions, which would be associated with research activity. Publications in eminent peer-reviewed journals are the main determinant of such academic activity. The quality of our publications needs to be improved; therefore, we must continue to conduct critical basic research that can be translated into clinical medicine and to advance ongoing clinical trials.

Research Activities

Upper gastrointestinal surgery

Advantages and disadvantages were intensely examined in each method of laparoscopic surgery for achalasia and reflux esophagitis, because the reputation of our technique enabled us to evaluate many patients despite these diseases being rare. Also, in surgery for esophageal cancer, hand-assisted laparoscopic-thoracoscopic surgery has been performed, and such advantages as less invasiveness and short hospitalization impressed many surgeons at various surgical meetings. Basic research in esophageal cancer led us to search for molecular markers indicating prognosis.

We have established a new technique of sentinel node navigation surgery without radioisotopes for patients with early gastric cancer using indocyanine green under infrared ray observation. A multicenter trial that evaluated our technique with an infrared ray laparoscopy systems was performed, and the rate, accuracy, and sensitivity of sentinel node detection were 100%. This method was, therefore, judged safe and efficient.

Colorectal surgery

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

Hepatobiliary and pancreatic surgery

The ongoing research activities in hepatobiliary and pancreatic surgery are as follows: 1) living donor liver transplantation (LDLT), regenerative medicine, and artificial liver (especially, implantable artificial liver), 2) chemotherapy for advanced pancreatic cancer; 3) expansion of surgical indications for hepatic resection in cases of multiple hepatic tumors; 4) laparoscopic surgery for hepatobiliary and pancreatic tumors; 5) development of a navigation system for intraoperative evaluation of biliary surgery; and 6) the significance and clinical application of the lipid mediator and high mobility group box-1 for hepatocellular carcinoma and liver diseases.

The first LDLT was successfully performed for a patient with cirrhosis and postnecrotic hepatocellular carcinoma on February 9, 2007. Our fourth LDLT was performed for a patient with primary biliary cirrhosis on July 25, 2008. All four recipients were discharged 19 to 32 days after surgery with a good clinical course. Our ongoing

research on regenerative medicine and artificial organs is expected to have a synergistic effect with liver transplantation medicine. We have performed a unified clinical trial for pancreatic cancer chemotherapy at our 4 university hospitals. Furthermore, at the university hospitals, translational research has been started into combination chemotherapy with gemcitabine and a naive protease inhibitor, FUT-175, which has the dual functions of nuclear factor κ -B inhibition and apoptosis induction in pancreatic cancer cell lines. Research activities 3, 4, 5, and 6 are ongoing after they were approval by the Ethics Committee of our university.

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

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

General Summary

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

Research Activities

Chest surgery

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

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

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

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

Our clinical studies are also evaluating new devices and methods, such as narrow-band imaging for the thoracoscopic diagnosis of benign and malignant lung diseases, and Laparo Sonic 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 expressionrelated advancement of the molecular genetic analysis of lung cancer, we are investigating whether 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.

Breast and endocrinology surgery

Cytotoxic chemotherapy is a standard treatment for breast cancer. However, responses to anticancer chemotherapy vary among individual tumors, and adverse reactions to therapy may outweigh the clinical benefits in some cases. Multicenter studies involving DNA microarray analysis are under way to seek effective drugs for individual tumors. 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 feasibility for standard use, especially after preoperative chemotherapy.

The usefulness of postoperative endocrine therapy for patients with hormone-receptor — positive breast cancer is recognized, but adverse effects, such as osteoporosis caused by aromatase inhibitors, must be considered. We are investigating the adverse effects of aromatase inhibitors, especially on bone mineral density, and comparing the effectiveness of therapeutic options in cases of decreased bone mineral density.

Various antihormonal agents have been used to treat hormone-receptor — positive breast cancer. We are evaluating several new antihormonal agents for patients with metastatic breast cancer refractory to previous antihormonal therapies.

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Department of Surgery Division of Pediatric Surgery and Vascular Surgery

Takao Ohki, Professor and Chairperson Yuji Kanaoka, Assistant Professor Atsushi Ishida, Assistant Professor Joji Yoshizawa, Assistant Professor

General Summary

Pediatric surgery

The division of pediatric surgery at The Jikei University Hospital is dedicated to providing expert surgical care for infants, children, and adolescents with congenital and acquired conditions. Year by year the expectations of patients' families for pediatric care become greater. They hope that operations for their children are less invasive. Our surgeons remain committed to the development of minimally invasive surgery to replace more invasive open procedures.

We created a new clinical system for pediatric surgery patients. We work in the outpatient offices of each of the four affiliated hospitals. All surgical outpatients who receive diagnoses at affiliated hospitals are introduced to and are operated on at the main hospital. After this system was established, the number of patients has been increasing. 1. Gastroesophageal reflex

In severe cases of gastroesophageal reflux, a surgical procedure called fundoplication may be performed. We have usually performed this procedure laparoscopically, which minimizes pain and shortens the postoperative recovery period. The number of handicapped children with gastroesophageal reflux has been increasing.

2. Pectus excavatum

The Nuss procedure aims to force the sternum forward and hold it there with an implanted steel bar. While less invasive than other procedures, the initial Nuss procedure surgery still requires 7 to 10 days of hospitalization for pain management. Our aim is to develop a new operative method that causes less pain.

3. Inguinal hernia

Inguinal hernia is the most common surgical problem of childhood. We use a new operation method called laparoscopic percutaneous extraperitoneal closure, which leaves no sutures on the surface of the abdomen.

Vascular surgery

1. Development of angiogenic therapy in ischemic disease

Angiogenesis, the process of postnatal neovascularization, is a critical component of several human diseases, including ischemic heart disease, cancer, diabetic microvascular disease, and rheumatoid arthritis. Moreover, angiogenesis is believed to be mediated by the proliferation, migration, and remodeling of endothelial progenitor cells (EPCs). Recent studies have shown that EPCs increase by direct injection of EPCs and stimulation with various cytokines and that administration of drugs have lead to improvement in ischemic diseases. We investigated the effects of new drugs or stem cells on an-

giogenesis in ischemic diseases.

2. Development of new vascular grafts and stents

The patency rate of small-caliber artificial blood vessels remains low, and the use of autologous venous grafts is inevitable; yet large venous autografts, such as the great saphenous vein, are often not available. Therefore, we are performing a study to develop new vascular grafts with intraluminally grown autologous vascular endothelial cells and with biodegradable materials. Moreover, we will develop biodegradable stents and drug-eluting stents.

3. Research on hemostatic fleece and closure devices in endovascular aortic aneurysm repair

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

4. Clinical study of specific antibodies against heparin-platelet factor 4 complexes Heparin is commonly used for anticoagulation in vascular surgery. Heparin-induced thrombocytopenia is a rare but life-treating complication with thrombosis of veins and arteries. Even if heparin use is limited, it occasionally induces the production of specific antibodies against heparin-platelet factor 4 (PF4) complexes. Patients with such antibodies are at increased risk for heparin-induced thrombocytopenia. The prevalence of these antibodies in patients receiving heparin is presumably underestimated. Accordingly, we prospectively measured antibodies against heparin-PF4 complexes and the activity of PF4 and investigated whether they are related, particularly in patients undergoing major vascular surgery. We measured these variables in 300 patients for 2 years.

The positivity rate was approximately 13% and was higher than expected. Moreover, the antibody-positive patients tend to have higher levels of PF4 activity than did antibody-negative patients. We are performing statistical analysis. The results of this study will be reported in 2008.

Research Activities

Pediatric surgery

1. Pediatric oncology

Despite intensive treatment, outcomes in cases of advanced malignant tumors in children have not been satisfactory. To improve treatment results, new approaches are essential. One approach for malignant tumors is the combination of gene therapy and molecularly targeted therapy.

2. Pediatric urology

Endoscopic treatment for vesicoureteral reflux might reduce the number of patients

referred for open surgery. Deflux (Q-Med AB, Uppsala, Sweden) is an injectable gel developed for the endoscopic treatment of vesicoureteral reflux. Deflux is the only agent approved by the United States Food and Drug Administration for this purpose. We are preparing to use Deflux for the endoscopic treatment of vesicoureteral reflux.

Vascular surgery

1. Therapeutic angiogenesis has emerged as a promising therapy for patients with ischemic diseases. Transplantation of bone marrow cells is reported to augment development of collateral circulation in ischemic organs either by differentiating into vascular cells or by secreting angiogenic cytokines. Recent evidence suggests that adipose tissues secrete several humoral factors and contain pluripotent stem cells. We evaluated the therapeutic potential of cells derived from adipose tissue to promote angiogenesis in cases of ischemia.

2. Plasma levels of high-density lipoprotein (HDL) are negatively correlated with the incidence of ischemic diseases. However, the molecular mechanism by which HDL prevents atherosclerosis is not fully understood. We investigated the effect of HDL on differentiation of EPCs and angiogenesis in ischemia.

3. There is an increasing need for functional small-diameter grafts for surgical revascularization. However, smaller vascular grafts made from synthetic biomaterials, particularly those smaller than 6 mm in diameter, are associated with a high incidence of thrombosis. Fibroin is a biodegradable protein derived from silk which provides an antithrombotic surface and serves as a scaffold for various types of cell in tissue engineering. We evaluated the potential of fibroin for creating artificial vascular prostheses for small arteries.

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Department of Orthopaedic Surgery

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

General Summary

Basic research

Recently, the basic research of the Department of Orthopaedic Surgery has focused on the functions of the main structural components of connective tissue, collagen, and proteoglycans, and studies to define clinically applicable biochemical markers is under way. We were the first research center in the world to suggest that collagen defects increase the risk of bone fractures; the idea provided the basis for research carried out by a Ministry of Health, Labour and Welfare study group and was incorporated into other large clinical trials. Furthermore, our concept that bone fractures are caused by abnormalities of the collagen structure was acclaimed by a Yale University professor in a *Nature*-related journal as an idea of great scientific importance which will inspire many research groups around the world.

In addition, successful results have been obtained from animal studies and human clinical trials in the field of reconstructive surgery, where the combination of growth factors and commercially available bone-grafting materials has been applied. Also in this field, some follow-up studies are being performed at other research centers, indicating that the road has been opened to the clinical application of the combined bone-grafting materials. Thus, the example of our department clearly indicates that relying only on research done by university graduates abroad plays a much less important role than does producing original study results, which can draw worldwide attention, lead to follow-up studies, establish world-acclaimed clinical standards, and thus define the "Jikei brand."

Clinical research

We believe that research at a cellular or experimental animal levels would lack meaning if it were not directly linked to clinical practice. As mentioned above, scientific concepts formulated at our department have already drawn worldwide attention and have led to follow-up studies and clinical trials.

Moreover, each study group within our department has developed metal plates or surgical devices intended to be directly applied to clinical practice. Furthermore, on the basis of the need to establish less-invasive arthroplasty methods and the rapid progress in this area, we have collected cases of computer-assisted arthroplasty. Submission of papers to international scientific journals is planned.

Research Activities

The learning curve of arthroscopic Bankart repair for anterior shoulder instability The learning curve for arthroscopic Bankart repair procedure was analyzed for a single surgeon. The procedure was performed for 50 patients with anterior shoulder instability; 1 patient had a recurrent dislocation. The data obtained indicated that 20 to 30 operations are required for an inexperienced surgeon to gain the skills necessary for the arthroscopic Bankart repair procedure.

Results of nonoperative treatment of complete dislocation of the acromioclavicular joint

We evaluated the conservative treatment of complete dislocation of the acromioclavicular joint in 20 athletes, whose Japanese Orthopaedic Association ACS and Symptom Severity Scale scores at the final follow-up examination were 85.6/90 points and 89.7 points, respectively. Two patients underwent surgical treatment. Conservative treatment for grade III dislocations was more efficient for athletes, because of better clinical outcomes and earlier recovery to return to play.

Distal radius fractures

Recent reports indicate the effectiveness of planar-side locking plates in the treatment of distal radius fractures. To increase the effectiveness of this method, we filled the fracture void with β -tricalcium phosphate (TCP), a bone-grafting material developed in our department, and obtained successful results. This material is now being applied to the repair of the distal radioulnar joint.

A distinctive bone metabolism in patients with neurofibromatosis type 1

Loss of neurofibromin, a molecular defect found in patients with neurofibromatosis type 1 (NF1), affects the skeleton and is implicated in parathyroid hormone (PTH)-related anabolic processes. In our study, levels of bone metabolic makers and PTH in the sera of 34 patients with NF1 were examined. Levels of PTH exceeded the normal range in 20.6% of patients; therefore, we concluded that high PTH levels occur more frequently in patients with NF1 than in the general population. Hence, our results suggest that subclinical hyperparathyroidism plays an important role in the development of abnormal skeletal features in NF1, although the underlying mechanism is not understood.

Advantages of the modular femoral stem for primary total hip arthroplasty in patients with developmental dislocation of the hip

Rotational alignments of the sleeve and neck parts of the modular femoral stem were evaluated at primary total hip arthroplasty in patients with developmental dislocation of the hip. Proximal sleeves were implanted at various rotational alignment angles ranging from 20° retroversion to 76° anteversion due to proximal femoral torsional deformity. In 69% of patients, it was necessary to implant the neck part at a rotational alignment different from that of the sleeve part to obtain sufficient hip stability. The degree of adjustment of rotational alignment ranged from a 50° decrease to a 45° increase.

The results indicate significant advantages of the rotation-adjustable modular stem for primary total hip arthroplasty in patients with developmental dislocation of the hip.

Computer-assisted total knee arthroplasty

With the advancement of surgical navigation systems (computer-assisted surgery) and their application in total knee arthroplasty (TKA), various perioperative analyses have become available. In our department, computer-assisted TKAs are performed and local soft-tissue tension forces during patellofemoral joint repair are intraoperatively measured at different angles by using a computer-assisted surgery — specific tension balancer. Furthermore, a cutting device allowing custom cutting profiles based on 3-dimensional magnetic resonance imaging (MRI) data is being developed. A comparative study on

joint alignment and efficacy of the device in future arthroplasty procedures is under way.

Development of osteotomy plates for hallux valgus

Hypermobility of the first ray is a predisposing factor for hallux valgus deformity. The results of our study suggest the first ray deviates dorsomedially during weight-bearing in patients with hallux valgus and that both the longitudinal and transverse arches become flat. To correct this deformity, 3-dimensional osteotomy of the first metatarsal is necessary. Because adequate fixing forces cannot be provided by conventional methods, specialized osteotomy locking plates are being developed. The configuration and size of the plates, the directions and number of screws, and osteotomy angles are being examined. Such plates would decrease the dependence on a surgeon's skill and, hence, improve operative results.

Granular cell tumor

The clinical course and pathologic features of granular cell tumor were studied. When MRI showed an unclear margin, histological examination usually demonstrated invasion of surrounding soft tissues. Thus, MRI is useful for preoperative planning; wide excision should be performed in locally aggressive cases.

Outcomes of bone giant cell tumor of the radius

All studied cases were classified as grade 3 according to the Campanacci classification. After aggressive curettage and adjuvant therapy with 99% ethanol, iliac bone grafting and plate fixation were performed. Recurrence was observed in 1 patient, in whom the same treatment was repeated without complications. The mean Enneking limb function evaluation score in all cases was 94%. The applied surgical method was useful as an initial treatment.

New approaches for assessing bone quality

The concept of bone quality is included in the Japanese Guidelines for Osteoporosis Prevention and Treatment. Evidence has accumulated that collagen cross-links play important roles in bone strength. We have demonstrated that in patients with osteoporotic fractures of the femoral neck, quantitative and qualitative deterioration of collagen cross-link formation might be affected by such factors as hyperhomocysteinemia,
oxidative stress, and vitamin B insufficiency. Additionally, we found that a functional polymorphism in the methylenetetrahydrofolate reductase gene locus, T allele (C677T), may be a risk factor for a future fracture. In a 5-year prospective study of Japanese women we found that a high urinary level of pentosidine is an independent risk factor for vertebral fractures.

Effects of alendronate on bone formation and osteoclastic resorption of beta-tricalcium phosphate

Resorption of beta-TCP is thought to involve both solution-mediated and cell-mediated disintegration. In our previous study, the mechanism of beta-TCP resorption was found to be based on cell-mediated disintegration by numerous tartrate-resistant acid phosphatase — positive giant cells. In the present study, 2 different experimental models were used, and the results showed that local application of alendronate reduced the number of osteoclasts on the surface of beta-TCP. Inhibition of osteoclast formation reduced beta-TCP resorption and, hence, enabled bone formation. Our results suggest that osteoclast-mediated resorption plays important roles in the resorption of beta-TCP and in bone formation.

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Department of Neurosurgery

Toshiaki Abe, Professor Shizuo Oi, Professor Satoshi Tani, Associate Professor Hisashi Onoue, Associate Professor Masami Kamio, Assistant Professor Hiroshi Mori, Assistant Professor Yoshiaki Miyazaki, Assistant Professor Satoshi Sawauchi, Assistant Professor Haruo Sakai, Professor Yuichi Murayama, Professor Tetsuro Kikuchi, Associate Professor Shigeyuki Murakami, Associate Professor Satoshi Ikeuchi, Assistant Professor Masato Nakajima, Assistant Professor Tatsuhiro Joki, 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.

Development of a new endovascular opening system

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

Development of a new endovascular operating system

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

Development of bioactive coils (Matrix coil)

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

Development of mebiol gel

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

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

Flow dynamics for intracerebral aneurysm

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

Brain tumor

In the therapy of malignant glioma, local recurrence often determines prognosis. The principal of therapy thus becomes the control of local recurrence. However, treating local recurrence with chemotherapy is difficult because the blood-brain barrier is a major obstacle preventing chemotherapeutic drugs from reaching brain tumors. To overcome these problems, a method has been developed for the local sustained release of chemotherapeutic agents by their incorporation into biodegradable polymers. The Gliadel Wafer (MGI Pharma, Inc., Bloomington, MN, USA), which contains carmustine, has been approved in Europe and America for the treatment of malignant glioma. On the

other hand, recent advances in liposome technology have shown promise relative to 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 for the treatment of malignant glioma. In our study we try to use doxorubicine within thermoreversible polymer for intracranial implantation, a strategy that has been shown to be safe and successful in the treatment of malignant gliomas. We will investigate the release kinetics, toxicity, distribution, and efficacy of this preparation *in vitro* and *in vivo*.

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

Neurotrauma

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

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

Syringomyelia

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

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

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

2. Electrophysiological research in patients with syringomyelia

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

3. Fluid in the syrinx

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

Division of Pediatric Neurosurgery

The Division of Pediatric Neurosurgery, The Jikei University Hospital Women's & Children's Medical Center, was established in October 2002. In the last 6 years more than 1,000 new cases of various entities have been collected and recorded in our data bank, including hydrocephalus (27% of cases), spina bifida (25%), brain tumors (13%), and craniofacial anomalies (8%). Since April 2003, clinical research fellows, 12 from other domestic universities and 9 from other countries (including Germany, Italy, Austria, Jordan, and Bulgaria) have taken part in our research activities.

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

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

A member of our department has been nominated as the chairman of the National Study Group on Spina Bifida and has been promoting further nationwide and international cooperative studies on controversial issues in this field. In the field of craniofacial anomaly research, we have extensively applied the distraction method to Japan's largest series of cases; the clinical efficacy has been summarized, and our extensive work received the honorable prize of the International Society for Pediatric Neurosurgery, Raimondi's Award in 2004, and the Kawabuchi Award in 2005.

Our clinical and research activities have been well maintained both in Tokyo (The Jikei University Hospital Women's & Children's Medical Center) and in Hannover, Germany (the International Neuroscience Institute) on the basis of firm international collaboration with world-leading pediatric neurosurgeons and related research workers. Our department has continued as the headquarters of the International Study Group on Neuroendoscopy, the Japanese Society for Pediatric Neurosurgery, the Japan Academy of Hydrocephalus Research, and a member of our department has served as the President of the Executive Board Committee of the International Society for Pediatric Neurosurgery.

gery and the Japan Association of Medical English Education.

Spine and spinal cord group

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

As a clinical research activity, the analysis of pain in patients with neuropathic pain has started. The Dyna CT scanning system in operating rooms 4 and 5 seems to be one of the most sophisticated and unique image-guided surgery systems, especially when used with a navigation system.

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

Hypothalamopituitary disease

In 1995, we developed the endoscopic endonasal trasethmosphenoidal technique, a new operative approach for parasellar diseases, in collaboration with otolaryngologists. Using this method, developed on the basis of the theory and practice of functional endoscopic sinus surgery, we use only an endoscope without a nasal speculum to go through ethmoid sinuses via the nostril to reach the sphenoid sinus. Thereafter, we have continued to improve the safety and effectiveness of our operative method by introducing a navigation system for nasal sinus surgery and developing surgical instruments. Now we are working to establish technical standards in collaboration with several other institutions.

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

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

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Department of Plastic and Reconstructive Surgery

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

General Summary

Research in the Department of Plastic and Reconstructive Surgery is focused on 4 basic areas: 1) the etiology and treatment of craniofacial anomalies, 2) the etiology and treatment of hand and foot anomalies, 3) the mechanism of wound healing and the grafting of skin and bone, and 4) microsurgical transplantation. The staff of the department comprises surgeons representing virtually all areas of plastic surgery and clinicians from related disciplines. For this reason, the department provides the stimulating atmosphere necessary for productive research. The participation in research studies provides plastic surgery residents and postresidency fellows 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 craniosynostoses Apert syndrome, or acrocephalosyndactyly, 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 craniosynostosis syndromes. Four types of monoclonal antibody, which respond only to the peptides derived from mice with mutation in Pro 253 Arg, have been successfully prepared.

Gene transfer into limb bud using electroporation technique

Electroporation was used to transfer genes into the extremities of cultured mammalian embryos. Ell Std-ddy mice were anesthetized with ether. Embryos, together with the placenta and embryonic membranes, were dissected from the surrounding decidua. The yolk sac, amnion, and chorioallantoic placenta were preserved in Hank's balanced salt solution. The pEGFP-N1 vector $(0.1 \,\mu I)$ was injected into the yolk sac. The extremities were grasped with forceps-type electrodes and electroporated with 3 pulses of 30 to 50 V for 50 milliseconds. After the amnion had been removed, the embryo was placed in a bottle filled with mouse serum solution. Ninety-five percent O₂ and 5% NO₂ were supplied to the bottle via a tube 4 times a day. The embryo was cultured at 37°C and rotated at 30 rpm for 24 hours. The placenta was removed, and the embryo was fixed in 4% paraformaldehyde. Frozen sections were prepared with liquid nitrogen and observed with fluorescent microscopy. Green fluorescent protein was observed throughout the entire body with 50 V and in more restricted areas with 40 V and 30 V. The embryos that were electroporated with 30 V showed gene transfer localized to the epidermis and dermis.

Distraction osteogenesis

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

Morphologic study of bone conduction mechanism

Fewer experiments of artificial bone osteoconductivity have involved the cranium than the extremities. An experimental study of osteoconductivity of β -tricalcium phosphate (β -TCP) in cranial bone defects was performed. Bone regeneration in full-thickness circular defects (10 mm in diameter) created bilaterally in the parietal bones of adult female Japanese white rabbits was evaluated. The animals were divided into 3 groups: in group A, a β -TCP disk (9.5 mm in diameter, 2.0 mm in thickness) was inserted into the bone defect; in group B, β -TCP granules (approximately 0.1 g) were inserted; and in group C, nothing was inserted. The periosteum was repaired, and care was taken to avoid damaging the dura. Bone regeneration was assessed with visual inspection, roentgenometry, and intensity and histological findings. In conclusion β -TCP has good biocompatibility with cranial bone.

Tissue engineering

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

Hemodynamic analysis of capillary blood vessels in patients with diabetes

The increase in the prevalence of diabetes has led to an increase in the prevalence of diabetic foot gangrene. Below-knee or above-knee amputation should be put off as long as possible by means of both conservative and surgical treatments. Other than the ankle-arm pressure index and the cardio-ankle vascular index, few effective methods for predicting diabetic foot lesions have been reported. Videomicroscopic analysis of blood flow through capillaries in the eponychium of the toes of patients with diabetes indicates the stage of microangiopathy and may predict diabetic foot lesions. The effectiveness of prophylactic treatment with a 5-hydroxytryptamine type 2A receptor

antagonist will be investigated with this examination system.

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Department of Cardiovascular Surgery

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

General Summary

The main investigations in our department involved clinical study, evaluation of changes in cardiac performance, and long-term outcomes of corrective surgery, and experiments to address clinical problems we are facing. Clinical investigations, including follow-up studies, of valvular and ischemic heart diseases, are a focus of our clinical research activities, as are studies of complex congenital anomalies. New treatment approaches taking advantage of new surgical techniques, devices, and research outcomes have been investigated and attempted. We are also performing several experimental studies with *in vivo* and *in vitro* models. The experimental projects include the application of autologous skeletal muscle as an assist device in heart failure, protection of the lung during extracorporeal circulation, and improvement of cardioplegic solutions during cardiac arrest. The major activities are described below.

Research Activities

Basic research

1. Studies of myocardial protection during open-heart surgery

1) Experimental studies of a new strategy for myocardial protection against ischemia/ reperfusion injury

On the basis of the results of a series of experimental studies of cardiac ischemia/ reperfusion injury in an *in-vivo* pig model, we established the efficiency and reliability of integrated intraoperative myocardial protection with modified St. Thomas' solution. Furthermore, a recent experimental study of hybrid cardioplegia (blood and crystalloid cardioplegia) demonstrated the critical importance of lowering Ca^{2+} content during blood cardioplegic reperfusion (terminal hot shot). More recently, we have performed experimental studies to examine the effect on reperfusion injury of a phosphodiesterase (PDE) III inhibitor added to the cardioplegic solution.

2) A high dose of PDE III inhibitor in terminal warm blood cardioplegia

To test the hypothesis that myocardial ischemia/reperfusion injury can be limited by adding a PDE III inhibitor to terminal warm blood cardioplegia (TWBCP), 25 infant piglets were placed on cardiopulmonary bypass (CPB) and subjected to 90 minutes of ischemia with a single dose of cold crystaroid cardioplegia, and reperfusion with or without TWBCP before aortic declamping. Left ventricular (LV) functional recovery assessed with LV pressure-volume loops with sonomicrometry and biochemical myocar-

dial injury evaluated with levels of troponin-T, creatine kinase (CK), and lipid peroxide were compared among group I (control without TWBCP), group II (low-Ca TWBCP), and group III (low-Ca TWBCP with the PDE inhibitor olprinone).

A significant improvement in cardiac function and a reduction in reperfusion-induced biochemical injury, associated with a marked suppression of the lipid peroxide level, were noted in Group III. On the basis of these results, we conclude that a high dose of a PDE III inhibitor in the TWBCP promotes rapid and sustained myocardial functional recovery and inhibits oxidative biochemical damage.

3) Efficiency of ischemic postconditioning: Reversal of myocardial injury after cardioplegic arrest with ischemic postconditioning during early reperfusion

This study tested the hypothesis that the myocardial damage induced by ischemia/ reperfusion can be reduced by ischemic postconditioning early during reperfusion.

Methods: Eighteen piglets weighing 10.3 ± 1.5 kg subjected to 90 minutes of ischemia with single-dose crystalloid cardioplegia followed by 60 minutes of reperfusion on CPB. In 12 of the piglets, the 2 types of ischemic postconditioning strategies — 6 cycles of 10 seconds of ischemia/reperfusion (protocol I) or 3 cycles of 30 seconds of ischemia/reperfusion (protocol I) — were applied before aortic unclamping, whereas the other 6 piglets were not treated (Control). The LV function (systolic/diastolic) was evaluated with end-systolic elastance (Ees) and the LV diastolic time constant during isovolumic relaxation (tau). Myocardial and blood levels of lipid peroxide, troponin T, and CK were measured.

Results: In the control group both systolic and diastolic LV dysfunction (depressed Ees: $54\pm14\%$ of preischemic value and increased tau ($240\%\pm38\%$) associated with oxidant-induced biochemical injury (increased CK, troponin, and lipid peroxidates) were noted after 90 minutes of cardioplegic ischemia followed by untreated reperfusion. In contrast, postconditioning, especially with protocol II, allowed significantly better LV functional recovery (%Ees: protocol I, $67\%\pm23\%$; protocol II, $130\pm43\%$; *p<0.01 vs. control group, $54\%\pm14\%$), tau (%tau: protocol I, $140\%\pm60\%$; protocol II, $123\%\pm43\%$; *p<0.01 vs. control group, $240\%\pm38\%$), and less myocardial biochemical injury (myocardial lipid peroxide: $123\pm21^*$; and $134\%\pm12\%$; *p<0.05 vs. 180 ± 34). Also, serum levels of CK, troponin, and lipid peroxide were reduced in both postconditioning groups.

Conclusion: Ischemic postconditioning during early reperfusion produces prompt myocardial functional recovery with decreased biochemical injury in an *in-vivo* piglet CPB model. The interval and duration of repeated brief ischemia/reperfusion during postconditioning might be crucial to determine the beneficial effects of ishemic postconditioning.

2. Clinical studies of the management of CPB in infants

1) Deleterious effect of hyperoxemia and the role of continuous infusion of human atrial natriuretic peptide

Because CPB simultaneously alters many factors, including cytokines, vasoactive mediators, and free radical generation, oxidative injury due to conventional hyperoxic CPB might occur in the infantile heart and lung. To test the hypothesis that the extremely high pO_2 levels during CPB induce oxidative cardiopulmonary changes, which can be modulated by continuous infusion of human atrial natriuretic peptide (hANP), we performed a clinical study to compare functional and biochemical effects after CPB in infants. There was no significant difference in respiratory or myocardial function or results of routine enzymatic evaluation between infants who were or were not treated with continuous infusion of hANP. However, continuous infusion of hANP had beneficial effects on cytokine generation and pulmonary vasoconstriction after CPB in infants with pulmonary hypertension. The present study suggests cardiopulmonary bypass causes a substantial inflammatory stress including oxidative lipid peroxidation and that these deleterious effects are at least partially prevented by continuous infusion of hANP

Congenital cardiac surgery

Clinical studies of pediatric heart surgeries

1. Fontan operation

Clinical studies reviewed the clinical records and data of patients who underwent staged univentricular repair, including the bidirectional Glenn procedure (BDG) and Fontantype operations, and 1) demonstrated the efficacy of the staged approach for high-risk candidates for the Fontan procedures and its indications; 2) examined risk analysis with a newly proposed index: the Fontan index; 3) assessed indications for the final Fontan conversion after the staged approach assessed by superior vena cava pressure during Glenn circulation; 4) clinical importance and limitation of the early volume-reduction strategy as an infantile surgical policy for Fontan candidates; 5) examined persistent hypoxia after BDG, and therapeutic management; and 6) involved the intraoperative evaluation of hemodynamic candidacy for the Fontan operation after BDG.

In 9 patients, in whom the staged Fontan procedure was indicated after BDG, we measured superior vena cava flow, which is equivalent to pulmonary artery flow in BDG physiology, by means of a transit-flow meter intraoperatively. Measurement of pulmonary artery flow and pulmonary vascular resistance, incorporated with serial volume loading, allowed the assessment of the pulmonary vascular reserve capacity in response to an increase in pulmonary flow to simulate Fontan circulation. In 4 patients in whom pulmonary artery flow had increased to as high as 2.01/min/body surface area under the acceptable range of central venous pressure (i.e., <15 mmHg), Fontan completion was successfully performed with excellent hemodynamic status.

2. Ross operation

The surgical outcome and long-term results of the Ross operation were reviewed, with a focus upon autograft durability, in 33 patients who underwent the Ross procedure from 1995 through 2007 with total aortic root replacement and pulmonary autografting. Autograft function was assessed with periodical echocardiographic evaluation postoperatively for as long as 12 years. There were no operative or acute deaths, but late reoperation was required for autograft regurgitation in 3 patients: (rate of freedom from reoperation for autograft failure: 87% over 12 years). Excellent durability of implanted pulmonary autograft valves was noted, especially in pediatric patients and in patients with preoperative aortic stenosis.

Adult cardiac surgery

Clinical studies

1) Patient-Prosthesis Mismatch: The Jikei Experience

Patient-prosthesis mismatch (PPM) was defined as being present "when the effective prosthetic valve area, after insertion into the patient, is less than that of a normal valve." However, on the basis of the correlation between the mean transvalvular pressure gradient and the corrected effective orifice area, PPM is currently defined as an effective orifice area divided by body surface area of $\leq 0.85 \text{ cm}^2/\text{m}^2$. The surgical procedure will differ for each patient because of variations in the size of the aortic annulus and because of the patient's age, sex, level of activity, level of motivation, and complications. However, the risk of PPM should always be minimized through the selection of appropriate surgical strategies, including aortic root enlargement, use of a supra-annular or high-performance prosthesis, and the use of a stentless bioprosthesis, aortic homograft, or pulmonary autograft. We reviewed our results for aortic valve replacement, when we routinely performed aortic root enlargement in patients younger than 65 years, while supra-annular implantation of a bioprosthesis (Carpentier-Edwards Perimount valve) was performed for patients 65 years or older with a small aortic annulus. Annular enlargement in active young adults may be contributing to longer survival and better quality of life. The smallest bioprosthetic valve (19 mm in diameter) rarely led to PPM in older patients, and the presence of PPM did not have negative effects on late survival.

2) Present and future aortic valve selection based on current guidelines

The choice of a mechanical or tissue valve to replace the aortic valve remains controversial. According to the guidelines of the American College of Cardiology/American Heart Association and the Japanese Circulation Society, based on recent reports of excellent long-term performance of bioprosthetic valves, we have chosen tissue valves as the most appropriate aortic valve for elderly patients. In patients younger than 65 years, tissue valves are becoming popular regardless of the second operation. The purpose of this study was to examine the appropriateness of our valve selection and to compare 12-year results after aortic valve replacement with mechanical and bioprosthetic valves in recent years. There was no difference in survival between the 2 groups. Few patients younger than 65 years would select a bioprosthetic valve after being informed of the advantage of an anticoagulation-free life. The main reasons for not using a bioprosthetic valve were the possibility of swine vesicular disease and the risk of reoperation. The clinical decision about the choice of aortic valve, based on the current guideline, would be appropriate and reasonable.

3) Mitral valve reconstruction: leaflet resection and suturing techniques

Mitral valve repair for mitral insufficiency is widely favored over valve replacement. We have enthusiastically performed resection and suturing of the prolapsed anterior and the posterior leaflets. We evaluated these results. The rates of freedom from reoperation at 10 years were $93\% \pm 5\%$ for triangular resection in the anterior leaflet and $96\% \pm 3\%$ for quadrangular resection in the posterior leaflet and did not differ significantly. Recently, we have performed triangular resection rather than quadrangular even for posterior and commissural lesions. This change completely eliminated late dehiscence

and leakage at the resected area by reducing annular stress.

4) Mitral valve ring

Prosthetic annuloplasty rings play an important role in mitral valve repair. This study was performed to evaluate the midterm results of the Carpentier-Edwards rigid ring, the Physio ring, and the Cosgrove band. From October 1991 through December 2005, 140 patients underwent mitral valve repair with the rigid ring (121 patients), the Physio ring (9 patients), or the Cosgrove band (10 patients). The mitral valve disease was degenerative in all patients. The Cosgrove band was used exclusively for posterior lesions. All patients were followed up with echocardiography. Sizes of rigid ring we have used ranged from 26 to 36 mm, with 30 mm being most common. Although the mitral orifice area was decreased after mitral valve repair in all patients, particularly in patients treated with the classic ring, no patients required reoperation because of mitral stenosis or left outflow obstruction (systolic anterior motion). Postoperative volume reductions of the LV at systole and diastole were observed. Ejection fractions were unchanged in all cases. The actuarial survival rate was $92.0\% \pm 3.0\%$ at 10 years. The rate of freedom from reoperation at 10 years was $96.0\% \pm 2.0\%$. By remodeling the enlarged annulus, the rigid ring has yielded promising midterm results in terms of the rates of survival and freedom from reoperation. However, use of a band may be reasonable if annular movement is desired.

5) Evaluation of a new portable device for measuring prothrombin time — international normalized ratio after cardiac surgery

We evaluated a new portable device (CoaguChek XP, Roche Diagnostics) for measuring the prothrombin time — international normalized ratio (PT-INR) in 63 patients after cardiac surgery and compared the results with those of the conventional method. There was a strong correlation between the PT-INR values measured conventionally and those obtained with the CoaguChek XP. This new device was easy to use, data were obtained rapidly, and the results were reliable. The CoaguChek XP will be particularly useful for outpatients. PT-INR self-management is expected to be introduced as soon as health insurance coverage is obtained.

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Department of Obstetrics and Gynecology

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

General Summary

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

Research Activities

Gynecologic oncology

1. CD147 (extracellular matrix metalloprotease inducer, basigin) plays important roles in tumor invasiveness and metastasis by activation of matrix metalloproteinases (MMPs). We showed that CD147 was expressed in 97.5% of endometrial carcinomas and in 83.1% of cervical squamous cell carcinomas. Especially in endometrial carcinomas, CD147 expression is correlated with various clinicopathological factors (stage, grade, lymph node metastasis, lymphatic vessel infiltration, distant metastasis, presence of ascites) and with recurrence-free survival. The aims of our study were to clarify the functional significance of CD147 in cancer progression and to examine the possibility of using CD147 as a novel biomarker or as a therapeutic target.

2. Genomic identification of significant targets in cancer analysis in ovarian carcinomas We analyzed somatic DNA copy number variation in various histological types of 78 ovarian carcinomas using genomic identification of significant targets in cancer analysis. Regions that were significantly (p-value <0.01) more common in serous tumors, after corrections for multiple hypothesis testing (indicating nonrandom distribution across subtypes), included (in order of significance): 8p23.3, 6q24.3, 11p15.5, 8p21.2, 16q22.1, 22q13.31, 4q22.1, 5q22.2, 7p22.3, and 14q24.2. Although no regions of copy number variation were significantly associated with any histological subtype other than serous tumors, an overrepresentation of amplification of 20q13.2 (ZNF217), 17q12 (CCL4), and 8q13.2-q21.11 (NCOA2) was observed in clear-cell tumors, and amplification of 1q21.3 (CTMP) and 1q42.13 (RAB4A) was observed in endometrioid tumors.

3. Japanese ovarian cancer patients that can classify the presence of cancer. The information is similar to that of sera of patients with ovarian cancer in the United States because N-dimensional clusters built on United States sera spectra created cluster maps predictive of the Japanese samples. The performance of the model was better than that

of any of the existing single-biomarker assays, although truly useful models await a much larger sample size and the use of independent validation sample sets to demonstrate their robustness. These results encourage us to start a large-scale, multisite collection of sera from Japanese patients with ovarian cancer to develop a Japanese ovarian cancer serum profile assay.

4. MicroRNA (miRNA) expression profiles for cancers, including those of the lung, breast, stomach, prostate, and colon, were examined to investigate the involvement of miRNA in carcinogenesis. We are now investigating the roles of miRNA in the resistance of human ovarian cancer cells to paclitaxel. This research has significant implications for therapeutic strategies for overcoming cancer cell chemoresistance.

5. Mitochondrial ultrastructure-associated chemotherapy response in ovarian cancer A mitochondrial scoring system was developed in association with platinum sensitivity in ovarian cancer and was applied to clinical samples. Twenty-four cases were included in this study. Excellent correlation to chemosensitivity was noted for 2 factors: electron density and distribution pattern. The total score of these 2 factors in 9 sensitive cases was 1.44 and was 3.58 in 19 resistant cases (P < 0.001). Receptor operative characteristics analysis revealed that the total cut-off score was 3 point. Scores with this system were strongly correlated with response, and this result suggests that this system would be of great value as a biomarker for chemosensitivity.

6. The prognostic factors in patients with advanced epithelial ovarian cancer have been identified by many investigators. We performed a study to construct a simple and powerful prognostic index (PI) of epithelial ovarian cancer (PIEPOC). The four prognostic factors that remained independently significant in the analysis were age, performance status, cell type, and size of residual disease. On the basis of the regression function, patients were classified into three risk groups, i.e., low risk (PI: 0–2), intermediate risk (PI: 3), and high risk (PI: 4–6). The PIEPOC was equally predictive in a validation sample (n=230), identifying 3 groups (5-year survival rate: 67% in the low-risk group, 43% in intermediate-risk group, 17% in the high-risk group).

7. Serum indoleamine 2,3-dioxygenase expression was positively correlated with impaired survival in patients with serous-type ovarian cancer.

We have previously reported that the indoleamine 2,3-dioxygenase (IDO) screened with the GeneChip is positively correlated with paclitaxel resistance and with impaired survival in patients with serous-type ovarian cancer. We established an enzyme-linked immunosorbent assay with an anti-IDO antibody for serum and measured serum IDO titers in 26 types of ovarian cancer. We compared the expression pattern in surgical specimens and the corresponding serum IDO titer and found a positive correlation in the serous type. These results suggest that the serum IDO level is a biomarker for seroustype ovarian cancer.

Fetomaternal medicine

1. Antiphospholipid syndrome (APS) is a clinical entity manifested by arterial and venous thromboses and recurrent miscarriages, and is caused by antiphospholipid antibodies. APS has also been observed in some complications of pregnancy, *e.g.*, pregnancy-induced hypertension, intrauterine growth restriction, and late fetal death.

However, little is known about how antiphospholipid antibodies (APLs) is involved in these complications. To be identified mechanisms of APLs in obstetrical complications by investigating the pathological significance, we established an experimental model for APS using wild type, Fc-knock-out mice and C_3 -knockout mice.

2. IDO plays essential roles in successful pregnancy and is induced by type 1 cytokines. The receptors of type 1 cytokines share a structure and a signal transduction pathway with prolactin. Because physiological levels of prolactin increase markedly during pregnancy, we examined a possible connection between prolactin and IDO expression. To investigate IDO expression in CD14 cells, we obtained mononuclear cells from 12 healthy persons and cultured the cells for 24 to 48 hours in the presence or absence of stimuli (interferon [IFN]- γ : 5,100 IU/ml; prolactin: 10, 100, or 500 ng/ml). IDO expression was up-regulated only when prolactin at physiological concentrations during pregnancy were present with 5 IU/ml of IFN- γ , whereas only prolactin and 5 IU/ml of IFN- γ were without effect. These findings suggest prolactin plays important roles in the maintenance of pregnancy, thereby up-regulating IDO, and support the view that the physiological concentration of IFN- γ is important for pregnancy.

3. Establishment of an immortalized human extravillous trophoblast cell line by infection with retroviral expression vectors

Studies of the function of human trophoblasts have been limited by a lack of suitable cell models. We aimed to obtain human normal trophoblast cell lines with long lifespans and consequently provide an ideal *in vitro* cell model. Primary human trophoblast cells were derived from the placenta of a woman undergoing elective abortion during the 7th week of gestation. The cells were immortalized by infection with retroviral expression vectors containing type 16 human papillomavirus E6 and E7 in combination with human telomerase reverse transcriptase. The characteristics of the cell line were analyzed. Immunocytochemical studies revealed staining for human chorionic gonadotrophin chain β , cytokeratin 7, HLA-G, and CD9 and an extravillous trophoblastic phenotype. Transwell insert invasion assay showed the invasiveness of this cell line, and gelatin zymography detected the secretion of MMP-2 and MMP-9. Karyotype analysis showed nearly normal choronosomal numbers with small deviations ranging from 46 to 48, and nude mouse assay showed no tumorigenecity. This newly immortalized cell line, HChEpC1b, will provide a useful model for the study of extravillous trophoblast function.

Reproductive endocrinology

1. Most of the mechanisms for achieving pregnancy have been clarified due to the development of assisted reproductive technololgies. However, the mechanism of implantation remains unclear.

CD147 is expressed at high levels on the surfaces of various tumor cells and stimulates MMPs. We hypothesized that CD147 plays an important role in implantation. The aim of this study was to determine the expression and hormonal regulation of the CD147 gene during the human implantation period in controlled ovarian hyperstimulation cycles. We found that CD147 and MMP-2 mRNAs in human endometrium during the

secretory phases were significantly decreased in controlled ovarian hyperstimulation cycles.

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Department of Urology

Shin Egawa, Professor Haruki Yamazaki, Associate Professor Koichi Kishimoto, Associate Professor Tetsuro Wada, Associate Professor Yasuyuki Suzuki, Assistant Professor Takashi Hatano, Assistant Professor Shoichi Onodera, Professor Isaho Ikemoto, Associate Professor Hiroshi Kiyota, Associate Professor Nozomu Furuta, Assistant Professor Koji Asano, Assistant Professor Kenta Miki, Assitant Professor

General Summary

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

Research Activities

Urologic oncology

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

- 1) Proteomics analysis of new biomarkers for prostate cancer and urothelial cancer
- 2) Gene therapy against urological malignancies
- 3) Research on prostate cancer stem cells

2. Clinical research: Several clinical studies are in progress at our institution. Results of some studies 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

Genitourinary tract infection

1. Basic research on antimicrobial resistance of *Neissseria gonorrhoeae*, especially resistance mechanisms in cephem-resistant *N. gonorrhoeae*

2. Clinical surveillance of gonococcal urethritis in the Tokyo metropolitan area

3. Clinical study of additive therapy with clarithromycin and cefteram for male gonococcal urethritis

4. Psychological education for chronic prostatitis/chronic pelvic pain syndrome

Neurourology and urodynamics

1. Study of dysuria

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

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

The frequency-volume chart

2. Study of nocturia

The relation between cardiac insufficiency and nocturia was shown by using brain natriuretic peptide levels and the frequency-volume chart.

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

Kidney and adrenal gland

1. Preoperative prediction with 99mTc-mercapto-asetyl-triglycine scintigraphy of renal function remaining after nephron-sparing surgery

2. Laparoscopic surgery for adrenal tumors

Endourology and extracorporeal shockwave therapy

- 1. Extracorporeal shockwave therapy for Peyronie's disease
- 2. Holmium-YAG laser ablation of the prostate for benign prostatic hypertrophy

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Reviews

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Department of Ophthalmology

Hiroshi Tsuneoka, Professor Keigo Shikishima, Associate Professor Genichiro Takahashi, Associate Professor Kazushige Toda, Assistant 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

General Summary

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

Research Activities

Color-vision defects and inherited retinal diseases

Our research goals are to investigate clinical features and the genetic basis of color-vision defects and inherited retinal diseases and to clarify genotype and phenotype correlations. The Lanthony desaturated panel D-15 test consists of 16 caps, similar to those of the Farnsworth dichotomous test. The color caps of the Lanthony test are of lower chroma and higher value than those of the Farnsworth test. We evaluated the Lanthony test in anomalous trichromats with congenital red-green color-vision defects. We examined 162 anomalous trichromats (155 male and 7 female) identified with a Nagel type I anomaloscope. All subjects had passing results on the Farnsworth test, 1.0 or better of best-corrected visual acuity, and no ocular disease. Pass/fail results of Lanthony test were compared between protanomalous trichromats and deteranomalous trichromats by means of the chi-square test (with significance at p < 0.05). Odds ratios (ORs) were calculated. The pass/fail results of the Lanthony test were compared between anomalous trichromats aged 7 to 10 years and those 11 years or older. Nineteen of the 54 (35.2%) protanomalous trichromats and 19 of the 108 (17.5%) deteranomalous trichromats had failing results on the Lanthony test. The difference was significant (OR, 2.50; 95% confidence interval, 1.1-5.7; p=0.013). The failure rate of anomalous trichromats aged 7 to 10 years was much higher than that of anomalous trichromats 11 years or older. The results suggest that failure on the Lanthony test is more likely for protanomalous trichromats than for deteranomalous trichromats and more likely for anomalous trichromats aged 7 to 10 years than for those 11 years or older.

Ocular oncology and histopathology

1. We reported 2 cases of conjunctival and corneal intraepithelial neoplasia treated by

topical fluorouracil and proposed the therapeutic regimen for this condition. We treated 2 patients with conjunctival and corneal intraepithelial neoplasia. One patient was a 73-year-old man, and the other patient was an 81-year-old woman. Both cases were unilateral. The diagnoses, by biopsy, were severe dysplasia and carcinoma *in situ*, respectively. After a trial period of treatment with 0.5% fluorouracil, the doses were changed to 1.0%, to be administered 4 times daily for 4 consecutive days. Treatment was repeated 6 times at intervals of 1 month. The lesions showed marked improvement in both cases. No corneal side effects developed. Pulsed treatment with topical fluorouracil is safe and effective for conjunctival and corneal intraepithelial neoplasia.

2. Malignant lymphoma in the eye consists of ocular adnexal lymphoma and intraocular lymphoma. A significantly increased incidence of malignant lymphoma in the ocular adnexa was found in our most recent series. An approach to the diagnosis of malignant lymphoma in the ocular region was reviewed.

Biochemistry

1. We investigated the protective effect of pigment epithelium-derived factor-loaded nanoparticles (PEDF-NPs) against photoreceptor degeneration in Royal College of Surgeons rats. Intravitreously administered PEDF-NPs showed significant protective effects, due to the targeting and the sustained release of PEDF *in situ*, against photoreceptor degeneration. The results of these studies suggest that the intravitreous injection of PEDF-NPs is a promising therapeutic strategy for rescue of photoreceptors and the retinal pigment epithelium.

2. Interleukin (IL)-31 is a newly discovered T-cell cytokine that, when overexpressed in mice, results in pruritus and skin dermatitis resembling human atopic dermatitis. Although IL-31 has been regarded as a factor in atopic dermatitis and bronchial asthma, its role in allergic conjunctivitis remains unknown. In this study, we investigated the effects of IL-31 on allergic conjunctivitis in a murine model of allergic conjunctivitis induced by Japanese cedar pollen allergen (Cry j 1). After sensitization with Japanese cedar pollen allergen (Cry j 1), IL-31 instillation stimulated conjunctival epithelial cells and activated macrophages in the conjunctiva. The (stimulated) epithelial cells produced such chemokines as thymus and activation-regulated cytokine and macrophage-derived chemokine, and recruited helper T type 2 cells into the conjunctiva. Finally, helper T type 2 cytokines induced eosinophil infiltration. In conclusion, the study revealed that IL-31 instillation following sensitization with Cry j 1 aggravates allergic conjunctivitis.

Eye movement

Binocular summation in the visual cortex was examined by comparing cortical responses of binocular and monocular visual stimulation by means of functional magnetic resonance imaging (fMRI). Two different checkerboard stimuli were used. The binocular condition demonstrated markedly greater signal intensities than did the monocular condition. Two stimuli demonstrated different binocular summation ratios and different increasing signal intensities. These results suggest that the binocular summation processes of these two visual stimuli differ in the visual cortex.

Neuro-ophthalmology

1. We examined performance characteristics of frequency-doubling perimetry (FDP) in comparison with standard automated perimetry (SAP) in patients with resolved optic neuritis. The Swedish interactive thresholding algorithm 30–2 program was used for SAP, and a full-threshold 30–2 program was used for FDP. Comparisons were made of the mean deviation, pattern standard deviation, and the percentage of abnormal points significantly depressed <0.5% in the total deviation probability plot. Defects detected with FDP were larger than those detected with SAP. The sensitivity of FDP for visual field defects in resolved optic neuritis is equal to and potentially greater than that of SAP. This short-term follow-up study in patients with resolved optic neuritis suggests that FDP detects characteristics of slower recovery in the fovea and in extrafoveal areas more effectively than does SAP. These properties may allow more accurate detection of visual field defects and may prove advantageous for monitoring patients with resolved optic neuritis.

2. We examined the association between the polymorphisms of the endothelial nitric oxide synthase (eNOS) gene and the occurrence of nonarteritic anterior ischemic optic neuropathy (NAION). There was no significant difference in the genotype distribution of the Glu298Asp polymorphism of exon 7 between patients with NAION and control subjects, whereas the genotype distribution of the T (-786) C polymorphism of the promoter region differed significantly. Subjects with the CC genotype of the T (-786) C polymorphism were more likely to have NAION than were subjects with the TT genotype. We found an increased prevalence of the T (-786) C polymorphism of the eNOS gene in patients with NAION. Our data suggest that the T (-786) C polymorphism of the eNOS gene is an important risk factor for NAION in Japanese subjects. 3. Pupillary abnormalities and the differential diagnosis of anisocoria were described in a textbook.

4. We reported a case of McCune-Albright syndrome presenting as acute compressive optic neuropathy.

5. Conventional magnetic resonance imaging cannot directly visualize wallerian degeneration of the optic radiation resulting from proximal axonal injury such as that caused by temporal lobe lesions. A new technique, diffusion tensor imaging (DTI), can show the extent of axonal conservation in the white matter. We performed fMRI and DTI simultaneously to explore the trajectory of the optic radiation and cortical activation in a patient with right-sided hemianopia and a temporal lobe lesion. Although the left occipital cortex was anatomically conserved, fMRI showed weaker cortical activation on the left side than on the right side. DTI tractography showed that the left optic radiation did not reach the occipital pole.

Our technique suggests that the hemianopia in this patient was caused by wallerian degeneration of the optic radiation. Both fMRI and DTI are useful for the clinical evaluation of cerebral visual disability.

Cornea and refractive surgery

The cornea group at The Jikei University chooses the most appropriate method of corneal surgery by discussing the various options with each patient. We replace only

those corneal components that are damaged. Photorefractive keratectomy (PTK) is effective for cases of surface corneal opacity, and automated lamellar therapeutic keratoplasty (ALTK) is also effective for cases of corneal stroma opacity. We also perform Descemet's stripping automated endothelial keratoplasty for corneal endothelium dysfunction.

We have adapted several new treatments for all corneal diseases, for example, xerophthalmia, corneal infection, corneal injury, hereditary corneal disease, allergic corneal disease, and keratoconus. ALTK, in which a microkeratome is used to create a lamellar flap, was performed in several cases of corneal opacity. We found that ALTK enables earlier suture removal and induces less astigmatism than does conventional lamellar keratoplasty. We studied the clinical outcomes of secondary implantation of iris-clip intraocular lens for aphakic eyes 5 years postoperatively. Clinically significant complications were not found with specular microscopy or laser flaremetry.

Glaucoma

1. We compared the effectiveness of Humphrey matrix perimetry, scanning laser polarimetry (GDx-VCC polarimeter, Carl Zeiss), and optical coherence tomography (OCT3000) for early detection of glaucoma. We found that the detection precision of Humphrey Matrix perimetry was equal to that of these optical imaging devices and that the pattern standard deviation was the most effective variable.

2. We evaluated from various aspects the shape and structure of blebs after trabeculectomy. We examined risk factors causing leaking blebs and overhanging blebs on the basis of operative method and postoperative management.

3. We investigated the effects of ocular optical system aberrations in perimetry, especially for peripheral vision measurement. In peripheral vision, there were astigmatism and coma aberrations, and the point images that were projected onto the retina differed depending on the measurement sites. These results suggest that the aberration affects the results of threshold measurements in the peripheral vision.

Electrophysiology

We are recording electroretinograms (ERGs) to evaluate retinal functional disorders in hereditary retinal degenerative diseases.

ERG waveforms are recorded as the responses of retinal cells, such as ganglion, amacrine, bipolar, and photoreceptor cells. For ERG recording, we use 3 types of machine: full-field, multifocal, and color. The full-field ERGs are recorded according to an international standard, and we record the responses from cones and rod cells separately. Multifocal ERGs are obtained from the central 30-degree area of the retina, reflect mainly cone function, and can record from 61 elements. Color ERG sseparately record responses to long-, middle-, and short-wavelength cones.

We plan to evaluate and analyze waveforms recorded from full-field ERGs. Moreover, as we extract 1 waveform from 1 type of retinal cell, we will attempt to detect retinal disorders at more minute levels.

Diabetes and vitreoretinal diseases

We have used 23-gauge and 25-gauge transconjunctival vitrectomy systems to treat macular holes, the epiretinal membrane, macular edema, and rhegmatogenous retinal detachment. The 25- and 23-gauge sutureless vitrectomy techniques decrease surgical trauma and increase 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 select cases that do not require the full capability of conventional vitrectomy. We plan to evaluate changes in regular and irregular corneal astigmatism after 25-gauge and 23-gauge transconjunctival sutureless vitrectomy.

Uveitis

1. A novel therapy with infliximab, a chimeric antibody against tumor necrosis factor α , in Behçet disease.

Intravenous infliximab significantly decreased the frequency of ocular attacks and improved visual acuity.

2. We identified bacterial and fungal pathogens in the intraocular fluid in endophthalmitis by means of DNA microarray analysis. The novel molecular approach can be performed, from pathogen isolation to species identification, within 12 hours in a research laboratory. We suggest that DNA microarray analysis is a useful tool for rapid identification of bacterial and fungal pathogens in the intraocular fluid in endophthalmitis.

3. We reported the clinical course of a patient with atypical Cogan's syndrome and posterior scleritis.

Visual neuropsychology

1. Two temporal channels in human V1 identified with fMRI

We measured the responses to spatial uniform (Ganzfeld) luminance changes in the human visual cortex using fMRI. We presented various temporal modulation stimuli without spatial contrast patterns and found that the BOLD signal consisted of transient and sustained channel responses reported in previous psychophysical studies. We identified these 2 independent channels with linear model analysis and revealed that the relative contribution of these channels varies with eccentricities across V1.

2. The objective visual field map with fMRI

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

3. Neural plasticity of area V1

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

Low vision

We compared the visual acuity of patients with both low vision and brain injury by means of 2 methods of measurement and examined the degree of divergence. Each eye's visual acuity was measured with both Teller acuity cards and Landolt rings. We also examined the degree of visual field loss. The visual acuity measured with Teller acuity cards was significantly better than that measured with Landolt rings in patients with brain injury and eccentric viewing.

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

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Department of Otorhinolaryngology

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

General Summary

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

Research Activities

Otology

We have performed basic research on regeneration of the middle ear mucosa and research designed for clinical application and have used virtual-reality technology to develop a navigation system to make surgery safer. We have entered the data from cholesteatoma surgery cases into a database and have performed an analysis of the surgical cases, an assessment of the surgical procedures, an epidemiologic survey, and an assessment of surgical outcomes. A genetic analysis of patients with hearing impairment was performed in collaboration with Shinshu University.

Operations for middle ear diseases have been performed in approximately 200 cases annually. Cochlear implant surgery has also been performed in several cases annually, and favorable results have been achieved. Operations on the skull base for cholesteatoma lesions of the petrous portion of the temporal bone have been performed in collaboration with the department of neurosurgery, and the numbers of cases in which hearing and facial-nerve function were preserved have increased. Approaches via the posterior cranial fossa, middle cranial fossa, and labyrinth have been used in operations for acoustic neuromas.

Outpatient clinics for otitis media and hearing impairment are held every Monday afternoon; the principal activities are the examination and follow-up of patients and the management of postoperative data. Changes in total middle ear cavity pressure associated with transmucosal gas exchange are measured in relation to the period of tympanostomy tube insertion for exudative otitis media and are used to make decisions about the timing of tube removal.

In neuro-otology, analyses of the results of experiments to demonstrate a relationship between fingertip contact pressure and postural control have been performed with a dynamic analyzer that employs a force plate and analysis software. In addition, the causative semicircular canal on the affected side in patients with benign paroxysmal positional vertigo is identified with nystagmus tests using an infrared charge-coupled device camera or with electronystagmography, and physical therapy is performed.

Rhinology

We have performed prospective clinical studies of endoscopic sinus surgery for paranasal sinusitis and of the postoperative course. We have used an endoscopic intranasal approach to treat many patients with diseases of the skull base, including paranasal sinus diseases, meningocele, pituitary diseases, and cerebrospinal fluid leaks, and have assessed the advantages and disadvantages of minimally invasive surgery.

With the aim of expanding the indications for and improving the safety of endoscopic sinus surgery, we developed a system for the superimposed display of stereoendoscopic images and stereonavigation and have applied it clinically.

We have investigated the involvement of fungi in intractable eosinophilic paranasal sinusitis. *Alternaria* induces production of interleukins 5 and 13 and interferon γ by isolated peripheral blood mononuclear cells from patients with paranasal sinusitis, but no such responses have been observed in healthy subjects. In addition, we have shown that the aspartate protease secreted by *Alternaria* induces various immunological reactions mediated by the protease-activated receptor-2 expressed on the surface of eosinophils and airway epithelial cells. We have also continued research related to the involvement of staphylococcal superantigens. With the aim of identifying the pathomorphogenetic factors of nasal allergy, we performed gene expression analysis of fibroblasts cultured from the inferior turbinates of patients with perennial allergic rhinitis and of healthy subjects. A comparative study of associations between the phenotypes and clinical disease patterns showed that the pattern of expression of many genes differed according to whether allergy was present.

Head and neck cancer

The main treatments for head and neck cancer are surgery, radiotherapy, chemoradiotherapy, and radiotherapy combined with superselective intra-arterial chemotherapy. We treat patients only after considering such factors as the location of the primary cancer, disease stage, possibility of curative surgery, presence of complications, performance status, age, and social and family background in each case.

1. Surgery: To perform curative surgery with consideration for maximal preservation of function, we have been performing reconstructive surgery with free flaps (rectus abdominis flaps, free jejunal flaps, forearm flaps, and anterolateral thigh flaps) for patients with advanced cancer (50 to 60 patients annually). In addition, we perform partial laryngectomy in cases of recurrence after radiotherapy and cases of T2-T3 laryngeal cancer and make efforts to preserve the larynx.

2. Radiotherapy has mainly been conducted on an outpatient basis to treat early cancer cases. Chemoradiotherapy has also been performed with the oral anticancer preparation S-1.

3. We perform chemoradiotherapy, with cisplatin and fluorouracil (5-FU) and concomitant radiotherapy, and subsequently perform adjuvant chemotherapy. Eligible cases are cases of cancers of the oropharynx, hypopharynx, or cervical esophagus in which laryngeal preservation is impossible and cases in which curative resection is impossible. We are planning to assess the introduction of chemoradiotherapy with a combination of the oral anticancer drug S-1 and cisplatin or chemoradiotherapy with a combination of 3 drugs by adding taxotere to cisplatin and 5-FU.

4. Radiation therapy combined with superselective intra-arterial infusion chemotherapy is an effective treatment for patients with extremely advanced disease for which curative surgery is impossible, and its introduction is being considered. We are using narrow-band imaging endoscopy (narrow-band filter endoscopy) for the early diagnosis of cancers of the head and neck and are striving for early detection of superficial cancers of the oropharynx and hypopharynx.

Research on phonation and swallowing function

1. Phonosurgery

We perform day surgery in the outpatient clinic with a flexible fiberscope or perform laryngomicrosurgery under general anesthesia to treat vocal cord polyps, vocal nodules, and vocal cord cysts. We have introduced the latest microflap method for laryngomicrosurgery. We are performing research to enable stricter determination of surgical indications by making comparisons based on evaluations by means of preoperative and postoperative acoustical analyses, aerodynamic tests, and surveys.

We have been performing laryngeal framework surgery in cases of unilateral recurrent nerve paralysis in which there has been no improvement after atelocollagen injection day surgery.

2. Diagnosis and treatment of spasmodic dysphonia

Botulinum therapy is the treatment of first choice for spasmodic dysphonia and was approved by the University's Ethics Committee in December 2004. The number of cases has been increasing, and the results of treatment surgical treatment for cases in which botulinum therapy proves ineffective are being assessed.

3. Evaluation and treatment of swallowing disorders

Cooperation with other departments, such as the departments of neurology and rehabilitation, and teamwork with allied medical personnel, including nurses, are important for the evaluation and treatment of swallowing disorders. We evaluate patients on the basis of videoendoscopic examination of swallowing and video fluorography tests and have been assessing treatment strategies and conducting swallowing exercises.

Research on sleep apnea syndrome

The relationship between sleep disorders and physical diseases, including metabolic syndrome, has been attracting attention. In children, attention has been drawn to the fact that sleep disorders affect the development of brain functions, including emotions, as in children who suddenly have tantrums. The aspects we have been studying include: the stability of nose breathing and sleep, sleep disorders in allergic rhinitis (pollinosis), attention deficit-hyperactivity disorder-like manifestations, the physical development of children with obstructive sleep apnea syndrome, and new surgical treatments for adults with obstructive sleep apnea syndrome.

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Department of Anesthesiology

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

General Summary

The year 2007 was the third academic year that Shoichi Uezono, M.D., served as chairperson of the Department of Anesthesiology. Our department provides a wide spectrum of medical care, from conducting preoperative anesthesia assessment to performing the most advanced life-support techniques in all perioperative settings, such as cardiac surgery, thoracic surgery, vascular surgery, interventional neuroradiology, comprehensive pain management, postanesthesia care unit, and the intensive care unit (ICU). The Department continues to grow in all these areas.

In 2006, Dr. Taniguchi was recruited to lead the clinical anesthesia research program. She was trained as a clinical research fellow in both Europe and the United States. She also has extensive experience in conducting phase II/III drug trials in Japan.

Research Activities

Research continues to grow as an important component of the Department's activities. Research opportunities are available in a variety of areas, including cardiac anesthesia; vascular anesthesia; interventional neuroradiology; pediatric anesthesia; acute and chronic pain management; critical care medicine; and medical informatics. Members of the department continue to be invited as visiting professors and guest lecturers at national and international institutions and meetings.

Below listed are the ongoing research projects in which the principal investigators are members of the Department of Anesthesiology.

Clinical research

1. Drs. Uezono and Hidano have been interested in outcome research in pediatric anesthesia. Their main goal is to determine the prevalence of congenital heart disease in patients with hemifacial microsomia. They hope to determine the role of neural crest cells in the development of the jaw and heart by means of gene analysis in affected individuals.

2. Dr. Shoji and his colleagues continue to search for better methods of managing postoperative pain. They have demonstrated several benefits of epidural patient-controlled analgesia over conventional intravenous drugs.

3. Dr. Kase has developed a new method to detect small amounts of endotoxin which are not detectable with conventional methods. His next effort is to determine whether extremely minute amounts of endotoxin have clinical significance in ICU patients.
4. Dr. Uchino and his colleagues examined, using the large database of ICU patients, the effect of a semiclosed ICU on the outcome of ICU patients. By introducing designated ICU physicians, the outcome data revealed some improvement.

5. Dr. Kitahara and his colleagues in the Pain Clinic have been interested in neuropathic pain after mastectomy for breast cancer. They have found that antidepressant drugs are effective for treating postmastectomy pain. They also serve as members of a committee to establish guidelines for the use opiates in the treatment of chronic pain.

Basic science research

1. Dr. Uezono has established a pulmonary hypertension laboratory to investigate gene therapy (antisense nucleoside therapy) in experimental pulmonary hypertension. This year his Grant-in-Aid for Scientific Research (Kakenhi) for this research was renewed. 2. Dr. Kondo and his group continue to study the effect of dexmedetomidine on the release of substance P in the spinal dorsal horn after tissue injury. Their results suggest the spinal cord is an important site for alpha 2 agonists to work as pain relievers.

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

Publications

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Davenport P, Tipping P, Boyce N, Bellomo R. A pilot study of high-adsorption hemofiltration in human septic shock. Int J Artif Organs 2007; **30:** 108-17.

Reviews

Uchino S. Choice of therapy and renal recovery. Crit Care Med 2008; 36: 238-42.

Department of Rehabilitation Medicine

Masahiro Abo, Professor

Kazushige Kobayashi, Associate Professor

General Summary

The main research projects in our department have been focused on neurorehabilitation, stroke, brain injury, and cognitive science.

Research Activities

Ability for basic movement as an early predictor of functioning related to activities of daily living in stroke patients

Background: Early functional outcomes for activities of daily living after a stroke may enable clinicians to establish treatment-optimal training and goals. The objective of this study was to assess at the bedside the relationship between the ability to perform basic movements, which were measured using a new scale, the Ability for Basic Movement Scale, in the early stage after stroke and functional ability at discharge from the hospital.

Methods: A total of 142 stroke patients participated in this prospective study. In addition to the Ability for Basic Movement Scale score, other predictor variables examined were age, limb paresis as indicated with the Brunnstrom stage, and functional ability as measured with the Barthel Index.

Results: Pearson correlation coefficient analysis showed that the state of functional ability at discharge was positively correlated with the total score of the Ability for Basic Movement Scale and with the Brunnstrom stage at all stages of data collection. The results of linear stepwise regression analysis indicated that significant predictors (75.6%) of functional ability at discharge were "turn over from supine position," "remain sitting," and "sit up" of the Ability for Basic Movement Scale 10 days after onset; age at onset of stroke; the baseline Barthel Index; and the baseline Brunnstrom stage.

Conclusions: This study provides evidence for the predictive value of the Ability for Basic Movement Scale with regard to functional ability in stroke patients.

Cerebral blood flow in patients with diffuse axonal injury: Examination of the easy Z-score imaging system utility

To evaluate the utility of the easy Z-score imaging system (eZIS) in cases of diffuse axonal injury (DAI), 27 patients with DAI were examined with magnetic resonance imaging (MRI) T2*-weighted sequence and with eZIS (7 women and 20 men; age range, 19–35 years; median age, 26.6 years). In this investigation, we excluded patients who had complications, such as acute subdural hematoma, acute epidural hematoma, intracerebral hematoma, and brain contusion. We performed neuropsychological tests and correlated the results with the findings of MRI/eZIS. Furthermore, we evaluated the degree of ventricular enlargement with the bifrontal cerebroventricular index (CVI).

Patients were divided into 2 groups: those with ventricular enlargement (bifrontal CVI>35%, 12 patients) and those without ventricular enlargement (bifrontal CVI<35%, 15 patients). Neuropsychological testing showed that all patients had cognitive deficits. Findings were abnormal in 15 of 27 patients with MRI T1/T2-weighted images and fluid attenuated inversion recovery, in 22 of 27 patients with MRI T2*weighted images, and in 24 of 27 patients with eZIS. On MRI T2*-weighted imaging, abnormal findings were present in the white matter of the frontal lobe, corpus callosum, and brainstem. With eZIS, blood flow degradation was found in the frontal lobe in 22 patients (81.5%) and in the cingulate gyrus in 12 patients (44.4%). Patients with ventricular enlargement had significantly lower scores on the Functional Independence Measure, Mini-Mental State Examination, Verbal IQ/Full Scale IQ, Trail Making Test-B, and Non-paired Miyake Paired Test. Among the 12 patients without ventricular enlargement who had no abnormal findings on MRI T1/T2-weighted images or fluid attenuated inversion recovery, 7 patients had abnormal findings on MRI T2*-weighted imaging and 10 patients had abnormal findings on eZIS. Results of MRI alone cannot fully explain DAI frontal-lobe dysfunction. However, the addition of eZIS-assisted analysis derived from single photon emission computed tomography data enabled us to determine where blood flow was decreased, *i.e.*, where neuronal functions conceivably might be reduced.

Activation of the prefrontal cortex during the Wisconsin Card Sorting Test (Keio Version) as measured with 2-channel near-infrared spectroscopy in patients with traumatic brain injury

To investigate brain activation in the prefrontal cortex during the Wisconsin Card Sorting Test (Keio Version) (KWCST), we examined changes in total hemoglobin volume in 8 patients with traumatic brain injury (TBI) and 20 healthy control subjects by means of 2-channel near-infrared spectroscopy. We found that the average total hemoglobin volume in the right prefrontal cortex during KWCST in patients with TBI $(-0.131\pm0.127 \text{ was significantly lower than that in control subjects (0.016\pm0.135 2× 3 ANOVA; p<0.05).$ These results demonstrate that patients with TBI have lower hemoglobin circulation in the right prefrontal cortex during the KWCST than do control subjects.

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by 2-channel near-infrared spectroscopy in patients with traumatic brain injury. *Eur Neurol* 2008; **59:** 24–30.

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Horiuchi H, Hashimoto K, Uruma G, Fujita G,

Soga T, lizuka H, Miyano S, Abo M. Successful rehabilitation approach after hip fracture to a

patient with above-elbow amputation. Am J Phys Med Rehabil 2007; 86: 861-4.

Department of Emergency Medicine

Takeki Ogawa, Professor Joji Ohtsuki, Associate Professor Tsutomu Koyama, Professor Satoshi Takeda, Assistant Professor

General Summary

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

Research Activities

- 1. Director of Japan Neurotrauma Data Bank Committee
- 2. Prognostic value of heart fatty acid binding protein for patients with chest symptoms in the emergency room
- 3. Research committee on higher cerebral function after traumatic brain injury

4. Research committee on impact biomechanics in automobile accidents (Society of Automotive Engineers of Japan, Inc.).

5. Published a revised edition of *Guidelines for the Treatment and Management of Severe Head Injury* (The Japan Society of Neurotraumatology).

6. Research group on Traumatic Intracranial Hypotension.

7. Supervision and development of ultrasound devices in the diagnosis and treatment of cerebrovascular disorders.

- 8. Secretary of research group on surgical maneuver for trauma.
- 9. Management course of Japan Advanced Trauma Evaluation and Care.

Publications

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T. Cardiopulmonary resuscitation education for our hospital staffs (3rd. Report) (in Japanese).
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Okuno K, Ogawa T. Skull fructure (opend, closed), Optic canal fructure; Trauma (in Japanese). *Handbook of Neurosurgical Emergency* 2008: 204–8.

Department of Endoscopy

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

General Summary

Our main fields of research are clinical studies of endoscopic diagnosis and the treatment of gastrointestinal (GI) and hepatobiliary-pancreatic diseases. In addition, we performed basic research for the development of novel instrumentation, image processing and analysis, and optical apparatuses, such as autofluorescence imaging (AFI), narrow-band imaging (NBI), supermagnified images, confocal endoscopy, and high degree of freedom therapeutic endoscopes. Published achievements and recent reports are summarized below.

Research Activities

Pharyngeal, esophageal and gastric malignancies

1. Endoscopic diagnosis of esophagogastric neoplastic lesions

Early detection and accurate diagnosis of esophagogastric premalignant and malignant lesions are essential for selecting the most appropriate therapeutic strategy for each patient. At our institution, the following novel optical technologies are used in addition to conventional white-light endoscopy. We have designed a series of prospective clinical research studies to evaluate and validate the benefits of the following novel imaging technologies. Most recently, we have introduced transnasal ultrathin endoscopy, which is expected to improve patient compliance, especially for screening in nonreferral hospitals, by reducing discomfort during the examination.

1) Magnifying endoscopic observation using an NBI system: This new diagnostic system consists of a magnifying (\times 80) endoscope and an NBI light source that provides detailed morphological information of capillaries on the mucosal surface. Our current study focus is to develop algorithms for the NBI technology, which may allow accurate analysis of the histological type of gastric carcinoma and the tumor extent without biopsy and allow early detection of precancerous changes in the specialized columnar epithelium of Barrrett's esophagus. The preliminary achievements have already been reported at several conferences and have been published. We have also introduced our own classification of Barrett's cancer based on magnified NBI findings and demonstrated its advantages over conventional diagnosis in a prospective study.

2) AFI: The AFI endoscopy system has recently been developed to visualize autofluorescence emitted from the wall of the GI tract. In theory, AFI will allow detection of premalignant lesions or early malignant lesions that lack a distinct endoscopic appearance. Although AFI is still associated with a high false-positive rate, we found that AFI in combination with conventional white-light imaging and NBI can improve

specificity.

3) Ultrathin endoscope (transnasal endoscope): The ultrathin endoscope can reduce discomfort during examinations. However, the image resolution of the ultrathin endoscope is lower than that of conventional endoscopes and, therefore, is associated with a higher false-negative rate. We found a higher false-negative rate for gastric lesions with the ultrathin endoscope than with a high-resolution endoscope. We are attempting to develop a method to study motility disorders with the ultrathin endoscope by assessing symptoms during the examination. Details of this motility study will be described in detail later.

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

2. Endoscopic treatment of esophageal and gastric malignancies

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

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

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

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

Many studies have shown an association between *Helicobacter pylori* infection and the development of gastric cancer. However, many aspects of this association remain unclear. This department, where endoscopic treatment of the gastric cancer is performed on a routine basis, is an ideal place to investigate this association. Studies of this association, especially of DNA methylation due to *H. pylori* infection, have been performed in cooperation with the Department of Gastroenterology, Toshiba General Hospital. We also have been exploring the roles of inducible nitric oxide synthase (iNOS) in the pathogenesis of *H. pylori* — associated diseases and have found that *H. pylori* eradication plays an important role in the repair process of methylated DNA and in the alteration of mucosal methylation in the 5 years after *H. pylori* eradication. The interim results have already been reported at several conferences and been published in Japan as well as internationally. In addition, we reported that the diverse topographical patterns of *H. pylori* — induced iNOS expression and iNOS gene polymorphism may contribute to the development of gastric cancer caused by *H. pylori* infection.

4. Diagnosis of oropharyngeal and hypopharyngeal malignancies

Endoscopic screening with iodine staining has allowed esophageal cancer to be detected at an early stage and has improved prognoses. However, metachronous or synchronous cancer in the oropharynx or hypopharynx has become the main factor affecting prognosis and the quality of life of patients with esophageal cancer. Although detecting such cancers at an early stage is important, performing chromoendoscopy for these cancers can be difficult owing to their location. Magnifying endoscopy performed in combination with the NBI system has enabled us to detect these hard-to-find cancers at an early stage without performing chromoendoscopy. A multicenter randomized controlled study of the clinical value of this new combination endoscopy has been performed.

Functional disorders of the upper GI tract

The etiology of GI reflux diseases, including nonerosive reflux disease and GI motility disorders, is difficult to determine. To understand the pathophysiology of these diseases and to development treatments for them, methods to evaluate the hypersensitivity and dysmotility of the GI tract should be established.

We developed a new method to evaluate esophageal function using a small-caliber endoscope. We started basic experiments to analyze the motility and sensitivity of the esophagus with the aim of applying this technique to clinical practice.

Diagnosis and treatment of esophagogastric varices

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

Enteroscopy and colonoscopy

1. Diagnostic techniques

Capsule endoscopy is a breakthrough modality that allows of the detection of diseases of the small intestine that cannot be reached with an ordinary endoscope system. In Western countries, capsule endoscopy has been performed for 300,000 patients and is recommended as a first-line examination for detecting diseases of the small intestine. Our department has been involved in a multicenter study of 12 major endoscopy centers to evaluate the usefulness of capsule endoscopy. We have also introduced a novel single-balloon enteroscope that allows interventions, such as biopsy and hemostasis, for lesions of the small intestine.

The prevalence of colon cancer has recently been increasing. Accurate preoperative evaluation of tumor invasion is essential for selecting the most appropriate therapeutic strategy for colonic lesions. To improve diagnostic accuracy, we utilize a magnifying endoscope with NBI/AFI technology.

2. Research on endoscopic intervention

Surgical resection has been the first choice of treatment for large sessile tumors of the colon. Recently, endoscopic *en bloc* resection with ESD, which is the standardized treatment for gastric lesions, has become an option for colonic lesions. However, endoscopic resection of large lesions in the narrow colonic lumen is technically challenging and has a higher risk of severe complications, such as perforation and bleeding. Our current efforts are focused on establishing a safe and reliable method for endoscopic removal of large colonic lesions and on applying ESD techniques to colonic lesions. Additionally, an infrared endoscopy system has been used to evaluate vessels on the ulcer base after ESD to prevent postoperative bleeding.

Pancreatobiliary endoscopy

1. Diagnosis of biliary and pancreatic diseases

Due to recent introduction of the diagnosis procedure combination, establishment of a standardized, systematic diagnostic algorithm for biliary and pancreatic diseases has become more important than ever. We are comparing the diagnostic accuracy for hepatopancreatic diseases of EUS-FNA, multidetector computed tomography, magnetic resonance cholangiopancreatography, and endoscopic retrograde cholangiopancreatography (ERCP). Additionally, we introduced a second-generation contrast medium for ultrasonic imaging for EUS diagnosis.

ERCP is a well-established procedure but is associated with risks of complications, some of which can be severe. We designed a new catheter to reduce trauma to the papilla, which is considered a major cause of pancreatitis after ERCP.

In the diagnosis of ampullary tumors of the duodenum, we perform detailed characterization of the mucosal surface structures using NBI with magnifying microstructures to determine if the lesion is benign or malignant. We also perform convex array EUS to evaluate the depth of tumor invasion. On the basis of these findings, we determine whether endoscopic papillectomy is indicated. Favorable clinical outcomes have been obtained so far.

2. Treatment using endoscopic techniques of pancreatobiliary diseases

A randomized, controlled study was performed to compare the usefulness of endoscopic sphincterotomy (EST) and endoscopic papillary balloon dilation (EPBD) for the removal of stones of the common bile duct. Data obtained from the comparative study are used for selecting the most appropriate treatment for individual patients with stones of the common bile duct. The appropriateness of the procedure selection has also been reviewed and discussed for further improvement of therapeutic results. Studies of long-term results, such as rates of recurrence and long-term complications, are under way. External biliary drainage (endoscopic nasobiliary drainage) and internal biliary drainage using a plastic stent (endoscopic biliary drainage) have been widely adopted for the treatment of obstructive jaundice. No criteria, however, have been established for selecting which of the two drainage methods should be used for individual patients. We are now performing a randomized, controlled study to compare the 2 different endoscopic therapeutic modalities. For cases of inoperable cancers of the bile duct or pancreatic head, we employ a metallic stent made of shape-memory alloy and performed a historical comparison with standard stents. Although EUS-guided celiac plexus block has been performed to control benign but persistent pain due to chronic pancreatitis, durability of the pain relief was short. Therefore, we performed EUS-guided celiac plexus neurolysis with injection of a small amount of ethanol and are evaluating its feasibility.

We also started animal experiments to develop new interventional technologies to achieve local control of pancreatic cancer.

Palliative care

More and more interest is being shown for palliative care. Various techniques have been developed to provide the best quality of life for critically or terminally ill patients.

Endoscopic procedures may play an important role, especially in supporting food intake. In our department, percutaneous endoscopic gastrostomy (PEG) is performed for patients who are unable to maintain sufficient oral intake. Although percutaneous endoscopic enterostomy (PEE) is conventionally not indicated for patients who have undergone gastric surgery, since 1994 we have extended the use of this procedure for such patients and have investigated its clinical usefulness. Kits for PEG that we developed have reduced the frequency of complications associated with PEE placement. To alleviate stenosis attributable to tumors of the GI tract and the bile ducts, we perform endoscopic ballooning/bougienage and subsequent metallic stenting. The therapeutic results have been good. To reduce the pain associated with chronic pancreatitis and inoperable pancreatic cancer, we perform transgastric celiac plexus block with EUS. These endoscopic procedures may greatly contribute to the improvement of the quality of life of patients who are not candidates for radical surgery. The cost-effectiveness of these interventions is another benefit.

Publications

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Reviews

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Department of Infection Control

Shoichi Onodera, Professor

Masaki Yoshida, Assistant Professor

Research Activities

Epidemiological research on sexually transmitted diseases

The Ministry of Health, Labour and Welfare's "Specific STD Injection Prevention Policy Promotion Study" Group has been operating, since 2006, with Professor Onodera as the lead researcher. This study group was formed to research, develop, and promote policies for preventing the emergence and spread of sexually transmitted diseases (STDs), according to the "STD Specific Infection Prevention Guidelines" that were revised in 2006. The study group's activities have included: 1) an epidemiological survey of STD emergence patterns; 2) experimental research for the early discovery and treatment of STDs in young adults; 3) the development of fast and accurate test methods for genital herpes and genital warts; and 4) drug-resistant gonococcus surveillance and the development of methods for diagnosing and treating gonococcal pharyngitis.

In Japan, the patterns of emergence of genital chlamydial infections, gonococcal infections, genital herpes infections, and genital warts (condyloma acuminata) are being investigated by means of a fixed-point survey. The selection of the designated reporting institutions in this fixed-point survey was left up to the individual local governments, and problems with this selection method have already been noted. The current study group investigated all cases of STDs in a limited geographical area to assess the validity of this fixed-point survey. In 2006, the 4 prefectures of Chiba, Ishikawa, Gifu, and Hyogo were designated as representative prefectures, and, in 2007, the 3 prefectures of Iwate, Ibaraki, and Tokushima were added; the cooperation of these 7 representative prefectures was requested in conducting a survey of all cases of STDs in each of these regions, in cooperation with the Japan Medical Association, the prefectural medical associations, physician's associations, and other bodies in each of the regions, to verify the validity of the fixed-point survey. Comparison of the fixed-point survey with this survey of all cases revealed a clear discrepancy between the 2 surveys in terms of the number of reported patients with STDs, particularly among young adults. In light of these results, we intend to propose that the study group reconsider the existing fixedpoint [method] and establish specific criteria regarding how the fixed points are selected.

Effect of silver-based antimicrobial agents for multidrug-resistant Pseudomonas aeruginosa

Nosocomial infections caused by multidrug-resistant *P. aeruginosa* have become a problem in recent years. In our daily lives, we come across many different kinds of antimicrobial products, including silver-based antimicrobials, which have excellent safety, durability, and practicality. We, therefore, investigated the antimicrobial effects of silver-based antimicrobial agents. *P. aeruginosa* (1,359 strains) was detected in 472

patients at The Jikei University Hospital from May 1, 2006, through March 31, 2007. *P. aeruginosa* exhibiting resistance to 2 of the 3 drugs imipenem, amikacin, and ciprofloxacin accounted for 137 of these isolates (10.1%) in 36 of these patients (7.6%). We then investigated 50 of these isolates (excluding those detected in the same samples or from the same patients). Muller-Hinton broth containing silver ions or nanocolloidal silver at various concentrations (0.5 to 64 ppm [mg/L]) was prepared and inoculated with 5×10^4 bacteria (50 strains) and, after 24 hours, was checked for the presence or absence of growth, and the minimum inhibitory concentrations (MICs) were measured. Of the *P. aeruginosa* isolates studied, 137 (10.1%) were observed to exhibit resistance to 2 of the 3 drugs imipenem, amikacin, and ciprofloxacin; the silver ion MICs with respect to 50 of these strains ranged from 1 to 8 ppm, and the nanocolloidal silver MICs from 1 to 4 ppm. Silver-coated filters inhibited bacterial growth and possessed an antimicrobial effect. The bacteria counts decreased over time in Muller-Hinton broth containing 4 to 20 ppm of nanocolloidal silver. They are expected to be useful for combating nosocomial infections caused by multidrug-resistant *P. aeruginosa*.

Clinical investigation of P. aeruginosa septicemia

Clinical and demographic factors, including age, sex, underlying diseases, and portals of entry, were investigated in 89 patients at The Jikei University Hospital from whose blood *P. aeruginosa* was isolated from April 2003 through December 2007, to investigate poor-prognosis factors for *P. aeruginosa* septicemia. Leukemia, present in 28 (31.5%) patients, was the most common underlying disease in the patients with *P. aeruginosa* septicemia, and the most common portal of entry was urogenital, in 20 (22.5%) of the patients. Of the 89 patients, 22 died within 30 days of the emergence of *P. aeruginosa* septicemia, for a mortality rate of 24.7%. Although no significant differences were seen in age, underlying disease, portal of entry, or other factors between patients who died and patients who survived, patients who died had significantly lower platelet counts and serum albumin levels than did surviving patients and were more likely to have blood cultures positive for bacteria other than *P. aeruginosa*. We also investigated the use of appropriate antimicrobial agents in the early stages of *P. aeruginosa* septicemia but obtained no evidence that such use improved the survival rate.

Study of patients receiving linezolid

We investigated patient characteristics and the clinical efficacy of linezolid at The Jikei University Hospital. The patients ranged in age from 0 to 80 years (mean: 66 years), and the reasons they received linezolid (multiple answers possible) were: previous treatment ineffective (10 patients); renal impairment (5 patients); and change of medication required due to adverse reaction(s) with previous treatment (4 patients). The clinical efficacy of linezolid was: effective (13 patients); ineffective (1 patient); and indeterminate (1 patient). Although mild anemia (in 4 patients), thrombocytopenia (in 3 patients), and hepatic impairment (in 1 patient) developed during treatment with linezolid, they resolved with conservative treatment. Linezolid is effective against drug-resistant gram-positive bacterial infections, even when existing drugs are ineffective or poorly tolerated, and also has an acceptable rate of adverse reactions.

because of concerns that the liberal use of linezolid could increase bacteria resistance, each patient should be examined carefully to ensure that linezolid is used appropriately.

Preventing outbreaks of norovirus gastroenteritis at medical institutions

We adopted the transcription-reverse transcription concerted (TRC) method, a new genetic amplification/detection method for nosocomial norovirus, and analyzed the results. Investigation of nosocomial outbreaks suggested that the TRC method is fast and is as sensitive as the reverse transcriptase-polymerase chain reaction. In addition, pediatric patients were observed in whom the virus was excreted over a long period of time. We concluded that, in the pediatric ward, close attention should be paid to measures for combating infection, even if symptoms have resolved.

Next, we investigated whether or not norovirus genes were present in the feces of medical professionals complaining of symptoms of gastroenteritis who were examined at The Jikei University Hospital. Of 123 medical professionals examined, 54 (43.9%) had positive results on the TRC NORO2 test and were, therefore, given a diagnosis of norovirus gastroenteritis. Of the 324 patients who were admitted during this same period, 90 (27.8%) had positive results, meaning that the rate of positive results on the norovirus test was significantly higher among medical professionals. Our results demonstrate the importance of monitoring the health of medical professionals.

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Department of Dentistry

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

General Summary

1. Clinical studies of temporomandibular disorders

We have continued studies of screening questionnaires and evaluation of quality of life for temporomandibular disorders (TMDs).

2. Basic studies of oral mucosal keratinocytes

We examined the expression and the function of nerve growth factor (NGF)/pro-NGF in normal oral mucosal keratinocytes (OMKs) *in vitro*. We also studied the *in-situ* immunohistological expression of antiapoptotic molecules, including NGF, in normal OMKs and in OMKs from patients with lichen planus.

Research Activities

Clinical studies of TMDs

1. Selecting and estimating the validity of question items for screening patients with TMDs

Background: There has been no questionnaire for epidemiologic study to assess the cross-validation of question items for screening TMDs. The aims of this study were to select question items for screening for TMDs and to access their validity.

Methods: We analyzed 2,360 dental patients (TMD prevalence: 11.9%) who visited 1 of 4 dental treatment facilities from October 2005 through September 2006. Cross-validation, criterion-based validation, and cut-off values for the accurate diagnosis of TMDs were determined.

Results: Four question items (five-point numeric rating scale) were selected from among 20 predetermined items by means of factor analysis and subsequent Mokken analysis. The validity was confirmed by means of construct-validity, cross-validity, criterion-related validity, polychotomous item response theory, and Cronbach's α . The cut-off value for the total value of the four items was 8.5, indicating a sensitivity of 0.746 and a specificity of 0.811.

2. Selection and validity test of one question item for the screening of TMDs

Background: There is no question item for testing the validity of screening for TMDs in dental patients.

Purpose: To select one question item from among four ready-made question items, which validated screening for TMDs in dental patients and to test the cross-validation and the diagnostic accuracy.

Methods and Subjects: A total of 1,245 dental patients (TMD prevalence: 12.4%) who visited one of four dental offices during one year were asked to answer four screening items with a 5-point numeric rating scale and a binary scale (yes/no). The patients

were randomly divided into two groups with SPSS software (SPSS, version 14, Tokyo). Items were selected with receiver operating characteristic curves and nonparametric dichotomous item scalability tests, and reliability was determined with calculations of diagnostic accuracy and Bayesian statistics.

Results: One question item, "when you open a mouth widely or close it or both, do you feel pain in your jaw?" was selected, and the cross-validation was proved. Measures of diagnostic accuracy were 0.701 (sensitivity), 0.871 (specificity), and 0.130 (false-positive rate). The false-positive group of question items included pericoronitis, dental caries, and periodontal disease, which would need to be diagnosed by means of close inspection.

3. Reliability of the pain-related limitations of daily function in the TMD questionnaire for Japanese patients with TMD

Background: We created a questionnaire survey to assess pain-related limitations of daily function in Japanese patients with TMDs (LDF-TMDQ) and reported various measures of its validity but did not examine its reliability.

Purpose: To examine the reliability of the LDF-TMDQ with the test-retest method.

Subject and Method: This study was performed as a part of a multicenter investigation of the polyphyletic evaluation and the polyphyletic treatment efficacy of TMD. The subjects were registered consecutively from among patients who visited to have treatment their problems to the Department of Dentistry, the Jikei University School of Medicine, as outpatients from January through December 2006. We used the LDF-TMDQ (1) database that was obtained from outpatients in the waiting room at their first visit. The LDF-TMDQ (2) database was obtained from patients with painful TMD who agreed to participate in this study on the same day (87 patients). In addition, we asked patients that they should fill out the LDF-TMDQ (2) that day after they returned home. The LDF-TMDQ consists of 3 latent variables: "Limitation in executing a certain task," "Limitation of mouth opening," and "Limitation of sleeping," and the questionnaire items were evaluated with a 5-point numeric rating scale. We analyzed reliability with the test-retest method, Spearman's correlation coefficient, Cronbach's α , and intraclass correlation coefficients in each group (SPSS, version 11).

Result: Spearman's correlation coefficients were 0.71, 0.69, and 0,77 (p<0.001); intraclass correlation coefficients were 0.83, 0,84, and 0.89 (p<0.001); and Cronbach's α was 0.89, 0.83, and 0.83 in "Limitation in executing a certain task," "Limitation of mouth opening," and "Limitation of sleeping," respectively.

Conclusion: Reliability in the same days of the LDF-TMDQ was recognized.

Basic studies of OMKs

1. NGF beta/pro-NGF and their receptors in normal human oral mucosa

Purpose: NGF- β and its precursor pro-NGF are important for the differentiation and survival of neurons and dermal keratinocytes. The aim of this study was to determine the role of NGF in the differentiation and wound healing of oral mucosa.

Results: Cultured normal human OMKs expressed mRNA for NGF- β /pro-NGF and for their receptors TrkA and p75^{NTR}. Lysates from cultured OMKs did not contain detectable amounts of mature 14-kDa NGF- β but did contain several NGF-proforms

with molecular weights of 32 to 114 kDa. Culture medium from OMKs contained 75-kDa pro-NGF, which was biologically active. The addition of NGF- β significantly enhanced the proliferation of OMK cultures and *in vitro* scratch closure. Immunostaining of biopsy specimens from normal oral mucosa showed pro-NGF in all epithelial layers. Staining for NGF was observed in the granular and upper spinous cell layers. Immunoreactivity for TrkA was detected in basal and parabasal cells, with weak to moderate immunoreactivity in spinous and granular cell layers. Staining for p75^{NTR} was seen in basal cell layers.

Conclusion: The findings indicate that NGF- β /pro-NGF have mitogenic and motogenic effects on OMKs and, therefore, may promote the healing of oral wounds. Differential expression of NGF and NGF receptors throughout the epithelium suggests a role in epithelial differentiation.

2. Survival signaling in OMKs of erythematous oral lichen planus

Background: OMKs in oral lichen planus are exposed to potentially cell-death-inducing factors, such as tumor necrosis factor- α and FasL, produced by the cells of the inflammatory infiltrate and by the OMKs themselves. Most lesions, however, do not show ulceration, the clinical manifestation of substantial keratinocyte death. The aim of this study was to find evidence that protective antiapoptotic mechanisms are activated in keratinocytes in a form of chronic oral lichen (erythematous oral lichen), simultaneously with the appearance of pathological cell-death signals.

Methods: Biopsy specimens from patients with normal oral mucosa or with erythematous oral lichen were compared by means of immunohistological staining.

Results: In OMKs from cases of erythematous oral lichen, both the proapoptotic Fas-associated protein with death domain and the antiapoptotic molecules p-IKK, nuclear factor- κ B/p50, Fas-associated death domain-like interleukin-1- β -converting enzyme-inhibitory protein, and cellular inhibitor of apoptosis 1 and 2 were strongly upregulated compared with OMKs from normal mucosa. There were no significant differences in the staining patterns for active caspase-3 or caspase-8, with only few cells being positive for both enzymes.

Conclusions: The observed marked increase in the expression of antiapoptotic molecules in erythematous oral lichen epithelium may counteract the proapoptotic assault and rescue the epithelium from rampant cell death and, thereby, clinical ulceration.

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Institute of DNA Medicine Department of Gene Therapy

Yoshikatsu Eto, Professor and Director

Toya Ohashi, Professor

Research Activities

Genetic disease

1. The main achievements of our laboratory this year are as follows.

1) Development of gene therapy for lysosomal storage disease: We generated recombinant lentivirus vectors expressing the enzymes missing in Pompe disease, Krabbe disease, and mucopolysaccharidosis type VII and administered the vectors to newborn model mice. In the case of Pompe disease, enzyme expression and a reduction in glycogen levels were observed for at least 4 months in the heart, which is main affected organ in this disease. Moreover, there was no immunological response against enzymes or viral vectors and no elevation of alanine aminotransferase.

2) Screening for Pompe disease among patients with muscular dystrophy: We screened for Pompe disease among patients in whom muscular dystrophy was diagnosed with dried blood spots. So far, Pompe disease has not been found in any of the 130 patients examined. We will continue this study in the future.

3) Establishment of induced pluripotent stem cells from patients with lysosomal storage disease: To understand the pathological mechanisms and the development of therapies for lysosomal storage diseases (LSDs), we successfully established various induced pluripotent stem (iPS) cells from a mouse model of an LSD.

4) Establishment of vascular endothelial cells from patients with Fabry disease: To understand the pathophysiological mechanism of Fabry disease, which affects the vascular system, we established vascular endothelial cells from patients with Fabry disease.

2. Diabetes mellitus: In vivo gene transfer of the cell cycle regulator gene in diabetic mice

Increased expression of p16^{INK4a} is a major cause for diabetes-associated inactivation of the cyclin D/cyclin-dependent kinase (CDK) complex which eventually reduces the islet beta cell mass in diabetes. We aimed to reactivate CDK4 to recover beta cell mass and glucose tolerance by transferring the CDK4^{R24C} gene, a variant that promotes the G1/S transition, to adult mice with diabetes. In R24C-treated mice the number of terminally differentiated beta cells was 250% greater than in sham-treated mice, and blood glucose levels were significantly lower. Restoration of aberrant islet structure by treatment with cell-cycle regulator genes is useful for regenerative medicine in diabetes. 3. Hepatocellular carcinoma

In our previous studies, we developed methods of gene therapy for hepatocellular carcinoma (HCC) and metastasis to the liver, which is an important prognostic factor for gastrointestinal cancers. In this study, we have explored a gene-therapeutic approach to stimulate antitumor immunity by adenovirus-mediated transfer of CD40

ligand to treat HCC and cancers metastatic to the liver. We demonstrated antitumor effects in both preventative and therapeutic models of HCC and liver metastasis. These studies suggest that cellular and humoral immunity contribute to this antitumor effect. Next, we will study orthotropic models of HCC and liver metastasis and then study the available clinical approaches via the hepatic artery.

Gynecologic oncology

1. Genomic identification of significant targets in cancer analysis of ovarian carcinomas We analyzed somatic DNA copy number variation in 78 specimens of various histological types of ovarian carcinomas using genomic identification of significant targets in cancer analysis. Regions with a p-value <0.01 after corrections for multiple hypothesis testing (indicating nonrandom distribution across subtypes) in order of significance included 8p23.3, 6q24.3, 11p15.5, 8p21.2, 16q22.1, 22q13.31, 4q22.1, 5q22.2, 7p22.3, and 14q24.2; these regions were more common in serous tumors. Although no regions of copy number variation were significantly associated with any histological subtype other than serous tumors, an overrepresentation of amplification of 20q13.2 (ZNF217), 17q12 (CCL4), and 8q13.2-q21.11 (NCOA2) was observed in clear-cell tumors, and amplification of 1q21.3 (CTMP) and 1q42.13 (RAB4A) was observed in endometrioid tumors. 2. Serum expression of indoleamine 2,3-dioxygenase was positively associated with impaired survival in patients with serous type ovarian cancer.

We have previously reported that the indoleamine 2,3-dioxygenase (IDO) screened with the GeneChip is positively associated with paclitaxel resistance and with impaired survival in patients with serous-type ovarian cancer. We established an enzyme-linked immunosorbent assay with an anti-IDO antibody for serum and measured serum IDO titers in 26 specimens of ovarian cancer. We compared the expression pattern in surgical specimens and the corresponding serum IDO titer and found an association in the serous type. These results suggest that the serum IDO titer may be a biomarker for the serous type of ovarian cancer.

Fetomaternal medicine

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

Investigation into the function of human trophoblasts has been limited by a lack of suitable cell models. We aimed to obtain human normal trophoblast cell lines with a long lifespan and consequently establish an ideal *in vitro* cell model. Primary human trophoblast cells were derived from a placenta obtained at elective abortion in the 7th week of gestation. The cells were immortalized by infection with retroviral expression vectors containing type 16 human papillomavirus E6 and E7 in combination with human telomerase reverse transcriptase. The cell line was characterized. Immunocytochemical studies revealed an extravillous trophoblastic phenotype with positive staining for human chorionic gonadotrophin chain β , cytokeratin 7, HLA-G, and CD9. Transwell insert invasion assay showed the invasiveness of this cell line, and gelatin zymography detected the secretion of matrix metalloproteinases 2 and 9. Karyotype analysis showed an almost normal chromosomal number with small deviations ranging

from 46 to 48, and nude mouse assay showed no tumorigenecity. This newly immortalized cell line, HChEpC1b, is a useful model for the study of extravillous trophoblast function.

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Institute of DNA Medicine Department of Oncology

Yoshikatsu Eto, Professor and Director Sadamu Homma, Associate Professor Tetsuro Kikuchi, Associate Professor Mikio Zeniya, Professor Junko Horiguchi-Yamada, Associate Professor

General Summary

Our research focuses on tumor immunology and leukemia cell biology. Basic investigations clarified the synergistic effects of a combination of chemotherapy and immunotherapy in cancer treatment. In clinical research, therapy with fusion cells (FCs) monocyte-derived dendritic cells mechanically fused with patient's tumor cells — continues, on the basis of the results of previous studies, for patients with brain tumors. The dendritic/fusion cell system also provides a mouse model to study the pathophysiology of autoimmune hepatitis.

Research Activities

New functional aspects of anticancer chemotherapeutic agents as activators of host antitumor immunity

Because the action of anticancer chemotherapeutic agents is based on their cytotoxic effects, it is inevitably accompanied by suppression of host antitumor immune activity. In contrast, some anticancer drugs elicit immune-activating effects represented by suppression of the activity of regulatory T cells. Our recent studies have demonstrated that the expression of major histocompatibility complex molecules, co-stimulatory molecules, death receptor molecules, and tumor-associated antigens on tumor cells is significantly up-regulated by treatment with some anticancer chemotherapeutic agents. Importantly, such immune-modulating effects are induced by low doses of these agents, which generate no cytocidal effects on tumor cells or immune suppression. These results would rationalize the possible synergistic effects of combined chemotherapy and immunotherapy for cancer treatment. A phase I clinical study of combination therapy with the WT1 peptide vaccine and gemcitabine for advanced pancreatic cancer is now being performed on the basis of synergism between immunotherapy and chemotherapy.

Clinical immunotherapy for brain tumors

Previous studies have revealed that subcutaneous injection of FCs is safe and elicits no adverse effects in patients. However, because a strong immunomodulator, interleukin 12, has become unavailable for clinical use, we are now combining FC therapy with chemotherapy to enhance the efficacy of the two strategies.

Adhesion-induced differentiation toward megakaryocytes in human leukemia cells Human leukemia JAS-R cells have megakaryoerythroid features. Some of the cells adhere to extracellular matrices and undergo dynamic morphological changes. Gene expression patterns compared between adherent and nonadherent cells confirmed these changes to be more megakaryocytic. Furthermore, most alterations of gene expression were also induced by fibronectin-derived Arg-Gly-Asp-Ser (RGDS) peptide, indicating that the integrin signal is relevant to these changes. These studies will disclose the mechanisms of the shift of megakaryoerythroid progenitors to megakaryocytes.

Role of regulatory T cells in the pathogenesis of autoimmune liver disease

We have previously reported that immunization of mice with dendritic cells loaded with well-differentiated hepatocellular carcinoma (HCC) cells followed by administration of recombinant interleukin 12 generated liver-specific inflammatory responses, inducing autoreactive cytotoxic T cells to normal hepatocytes by possible immune cross-talk between hepatocytes and HCC cells. This animal model is useful for analyzing the pathogenesis and pathophysiology of autoimmune liver diseases, such as autoimmune hepatitis. The role of regulatory T cells (Tregs) on the development of autoimmune liver disease was examined using this animal model. Abundant Tregs were observed to have accumulated in the liver at the peak of liver inflammation, whereas the number of Tregs in the spleen significantly decreased, suggesting that Tregs are recruited into liver inflammatory sites from the spleen. The expression of transforming growth factor-beta, a key cytokine for the induction of Tregs, and several Treg-associated chemokines in the liver were found to be enhanced by generation of liver-specific inflammation. These results suggest that Tregs have a homeostatic suppressive effect on autoimmune liver inflammation and that a functional defect of Tregs in patients with autoimmune hepatitis accelerate disease progression.

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Institute of DNA Medicine Department of Molecular Genetics

Hisashi Yamada, Professor and Director Masaharu Akiyama, Assistant Professor Takeshi Kawano, Assistant Professor

General Summary

Cancer is the leading cause of death in Japan. More than 300,000 people die of cancer every year. With the goal of conquering cancer, a state anticancer policy was established out in 2007. Under such a situation, we are studying to develop innovative therapeutic methods.

Research Activities

Exploring the etiology of human leukemia

Two groups of genes are involved in the pathogenesis of leukemia. One group of genes regulates the growth of hematopoietic cells, and the other controls the differentiation of cells. These genes co-operate to develop leukemias. Both megakaryocytic and erythroid leukemias are thought to originate from megakaryoerythroid progenitor cells, but the clinical characteristics of the leukemias differ markedly. Moreover, the molecular mechanism of lineage shifting remains largely unclear. We recently established a megakaryoerythroid leukemia cell line, JAS-R, whose cells show a lineage shift depending on culture conditions. The interaction between fibronectin and integrin elicits the megakaryocytic phenotype and leads to the loss of erythroid characteristics in JAS-R cells. Expression profiling analysis revealed that several transcription factors changed during lineage shifting. Of these genes, FLI1 was considered to be responsible for the expression of the megakaryocytic phenotype. These results are useful for understanding normal hematopoiesis and the treatment of leukemia.

Pharmacology of anticancer drugs

Several lines of evidences demonstrate that telomerase is a promising molecular target for cancer therapy. 5,10,15,20-Tetra-(N-methyl-4-pyridyl)porphyrin (TMPyP4) is a compound that binds to telomere DNA stably and tightly and blocks telomerase activity *in vitro*. Thus, we studied the anticancer activity of TMPyP4 using K562 leukemic cells and found that TMPyP4 directly inhibits the growth of K562 cells. We also studied whether TMPyP4 functions as an anticancer agent for other cancer cells. Two retinoblastoma cell lines, Y79 and WERI-Rb1, were studied. Apoptosis was induced in both cell lines by short-term exposure to TMPyP4. This apoptosis was associated with the phosphorylation of P53 and the activation of mitogen-activated protein kinase. Furthermore, pretreatment of Y79 cells with TMPyP4 increased radiation-induced cell death. These findings show that TMPyP4 is a potential anticancer agent and may be applicable for some patients with retinoblastoma who require radiation therapy.

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Institute of DNA Medicine Department of Molecular Immunology

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

General Summary

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

Research Activities

Interleukin 31 increases serum IgE levels in mice

Interleukin (IL)-31 transgenic mice that we have established show higher serum IgE levels than do their nontransgenic littermates. To confirm the association of IL-31 with serum IgE levels observed in the transgenic mice, recombinant (r) murine IL-31 was administered to normal C57BL/6 and BALB/c mice and to IL-4 receptor α -deficient knock-out mice. Serum IgE levels of mice were measured with a 2-site capture assay. To investigate the production of IL-4, IL-5, IL-13, and interferon γ , spleen cells from mice were cultured for 5 days, after which culture supernatants were harvested and tested for cytokine activity by means of sandwich enzyme-linked immunosorbent assay. Serum IgE levels of C57BL/6 and BALB/c mice that received injections of rIL-31 were significantly higher than those of mice treated with phosphate-buffered saline (PBS). However, no significant increase in serum IgE levels was observed in IL-4 receptor α -deficient knock-out mice that received injections of IL-31. Furthermore, production of IL-13 and IL-5 was significantly higher in the rIL-31-treated C57BL/6 mice than in PBS-treated control mice. In contrast, no significant difference in interferon γ production activity was found between rIL-31-treated mice and PBS-treated mice, whereas the concentration of IL-4 was below the detectable level in both groups. These results suggest that IL-31 is involved in the enhancement of serum IgE levels via induction of IL-13 expression.

Decreased numbers of CD4+ and CD8+ cells containing signal-joint T-cell receptor excision circles in patients with systemic lupus erythematosus

Patients with systemic lupus erythematosus (SLE) have decreased numbers of peripheral blood T cells containing signal-joint T cell receptor excision circles (Sj TRECs), which are considered an indicator of thymic output. The objective of this study was to investigate the mechanism of the decrease in such T cells. Peripheral blood T cells from patients with SLE were classified as CD4+ or CD8+ cells. Levels of Sj TREC were measured with the real-time polymerase chain reaction. Telomerase activity was determined with the telomeric repeat amplification protocol assay. The numbers of

CD4+ and CD8+ cells containing Sj TREC in the peripheral blood were lower in patients with SLE than in control subjects. A correlation was found between the number of Sj TREC-positive CD4+ cells and the number of CD8+ cells. The Sj TREC level is influenced by an increase in cell division. To examine this increase, telomerase activity, as an indicator of cell division, was measured simultaneously; however, there was no correlation between the Sj TREC level and telomerase activity. These results suggest that thymic output is decreased in patients with SLE.

Tenascin-C is required for proliferation of astrocytes in primary culture

Astrocytes in primary culture can be classified morphologically into 2 types: fibrous astrocytes and protoplasmic astrocytes. To examine the role of tenascin-C in an *in-vitro* astrocyte culture, primary cultures of astrocytes prepared from the brains of wild-type and of tenascin-C-deficient embryonic mice were analyzed. In primary culture of astrocytes from tenascin-C-deficient mice, fibrous astrocytes did not appear, and astrocytes did not become tile-shaped when they came in contact with each other. The rate of 5-bromo-2'-deoxyuridine incorporation in a cell proliferation assay was much lower for astrocytes from tenascin-C-deficient mice than for astrocytes from wild-type mice. These results suggest that tenascin-C is an essential molecule for maintaining the proliferation and proper morphology of astrocytes in primary culture.

Publications

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Institute of DNA Medicine Department of Molecular Cell Biology

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

General Summary

Our research aims are molecular analysis and visualization of cellular biological events under physiological and pathological conditions. To achieve these aims, the department has 2 different approaches: biochemistry and ultrafine morphology. Through a combination of these approaches, we try to explore medical life sciences.

Research Activities

Development of sonodynamic therapy for malignant glioma

Ultrasound has commonly been used as a diagnostic tool. It is handy, convenient, and less costly than other modalities. It is also relatively safe because it does not emit ionizing radiation and because many clinicians and medical technologists are familiar with this technology. In addition to its diagnostic application, therapeutic insonation has also been developed. One therapeutic application of ultrasound is sonodynamic therapy. When applied with agents activated by ultrasound, insonation induces cell death in nearby tissues. Using the method, we are trying to establish a new treatment for malignant glioma. The microbubble agent Levovist is used as an ultrasoundenhancer, and with such agents therapy and diagnosis can be simultaneously achieved as theragnosis.

Development of 3-dimensional cell culture

Cell culture is a basic tool for understanding the characteristics of tissues and organs in the human body. The procedure is also essential for the development of diagnostics and therapeutics for human diseases. However, vital cellular functions present in tissues or organs are missed by ordinary "flask" or "culture dish"-based cell cultures. From this point of view, we tried to establish culture methods that mimic human body environments. We proposed 3-dimensional (3-D) cell culture and investigated the morphology of human glioma cell lines. Bioadaptable and biodegradable gelatin was used as a scaffold for growth of glioma cells. The 3-D culture showed morphologic features that could not be observed in conventional cell culture. We conclude this culture method is useful for evaluating the characteristics of gliomas in the human body.

Establishment of a quick and sensitive endotoxin assay

Limulus amebocyte lysate (LAL) reacts with bacterial endotoxins to form gel clots. The gelation time for the entire LAL solution depends on the concentration of endotoxins in the sample. Although this end-product LAL test is convenient for estimating the level of bacterial endotoxins, the cascade reaction is extremely slow, and gelation does not proceed when the concentration of endotoxins is extremely low. The kineticturbidimetric test is another test commonly used to assay endotoxin levels, but in this end-point method small amounts of endotoxin cannot evoke sufficient gel-clot formation. In addition, although the kinetic-turbidimetric assay is convenient for estimating the concentration of endotoxins in clinical samples, the detection of the reaction at low endotoxin levels is time consuming. Both the end-product assay and the end-point assay also have problems regarding their sensitivity (a cut-off level of greater than 1 pg/mL). We have developed a different detection system for the LAL test that can directly detect the clotting enzyme product. The system is quick and sensitive, even in the presence of only small amounts of endotoxin. The gelation of LAL evoked by bacterial endotoxins can be detected earlier than with usual methods by using laser scattering photometry to recognize the formation of small particles of clotted enzyme produced when the reaction mixture is agitated. The appearance of these small particles means that the influence of endotoxins has stimulated activation of the clotting enzyme across the LAL cascade, and the timing of their appearance is related to the endotoxin concentration. This new method can be used as a quick and sensitive endotoxin assay. The average endotoxin level of healthy volunteers was assayed to be 0.0738 pg/mL (range, 0.0312-0.3445 pg/mL, n=11) within 70 minutes from the start of the assay.

Functional analysis of tight junctions

Tight junctions (TJs) in the epithelia and endothelia restrict the paracellular flux of water and solutes. In the epidermis, the significance of TJs is largely unknown because of the structural complexity of the epidermis. To understand TJ functions in the epidermis, a specific method for TJ disruption is needed. Sodium caprate is a well-known absorption enhancer which causes dilatations of TJs and increases paracellular permeability in the intestine. We investigated the effects of sodium caprate on cultured human 3-D skin to understand TJ functions in the epidermis. With sodium caprate treatment, transepidermal resistance decreased, indicating paracellular barrier disruption. The expression of claudin-1 and occludin increased, whereas their localization was fragmented. Immunoreplica electron microscopic images showed TJ strands were fragmented as well. These results suggest that C10-induced TJ disruption in the cultured human 3-D skin as well and this method can be applied to further study of epidermal TJ function.

Morphology and gene expression relationship in 3-D glioma culture

The relationships between morphological differences observed with scanning electron microscopy and gene expressions with the 3-D cell culture technique were evaluated with the real-time polymerase chain reaction and immunohistochemical analysis. Morphological differences observed with scanning electron microscopy of brain tumor cells in 3-D cell culture have been clarified. Four gliomas cell line — T98G, U118MG, A172, and KNS42 — were used for this study. Scaffolds for the 3-D culture were constructed from gelatin. Four cell-signaling — related genes, including those for transforming growth factor-beta (TGF- β) and vascular endothelial growth factor (VEGF), were

decreased. TGF- β and VEGF are involved in the positive or negative regulation of cell survival and stimulation. In particular, VEGF is associated with angiogenesis and regulates a process necessary for tumor growth. The cells in 3-D culture showed characteristic morphological differences and resulted in the down-regulation of different genes. The result suggests that gene expression is associated with morphology in 3-D culture; however, the expression of genes related to growth and survival is still under examination.

In another project, an attempt was made to conjugate nanoparticles with a monoclonal antibody, JT95, established by Takeyama *et al.* at The Jikei University School of Medicine. The antibody specifically reacts with human thyroid cancer. When labeled with nanoparticles, the antibody will improve the sensitivity and accuracy of antigen assays.

Publications

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Institute of DNA Medicine Project Laboratory for Kidney Regeneration

Takashi Yokoo, Director

General Summary

Many efforts has been made to apply regenerative medicine to clinical renal diseases, and some renal diseases in which the renal structure is maintained might be treated by infusing stem cells isolated from the bone marrow or the adult kidney. However, such a cell-therapy — based strategy cannot be applied to chronic renal diseases in which the renal structure, including scaffolding, has been completely disrupted. Therefore, the aim of research for absolute kidney regeneration should be to develop a way to rebuild an entire functional kidney de novo as a substitute for dialysis. However, because of the anatomical complexity of the kidney and the need for residential cells to communicate with one another to fulfill renal functions, the kidney has been considered the most difficult organ to regenerate. We are investigating the potential for reconstructing an organized and functional kidney structure, using the developing xenoembryo as an organ factory.

Research Activities

Establishment of an erythropoietin-producing organoid from human mesenchymal stem cells

Differentiation of autologous stem cells into functional transplantable tissues for organ regeneration is a promising regenerative therapeutic approach for cancer, diabetes, and many other human diseases. Yet to be established, however, is differentiation into tissue capable of producing erythropoietin, which has a critical function in anemia. Here we report a novel erythropoietin-producing organ-like structure (organoid) derived from human mesenchymal stem cells (hMSCs). Using our previously established relay culture system, an hMSC-derived, human erythropoietin-competent organoid was established in rat omentum. The organoid-derived levels of human erythropoietin increased in response to anemia induced by rapid blood withdrawal. In addition, when native (rat) erythropoietin production was suppressed, the presence of an organoid enhanced recovery from anemia. Together these results confirmed the generation of a stem-cell — derived organoid that can produce erythropoietin and is sensitive to physiological regulation.

Publications

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Department of Neuroscience Division of Neuropathology

Satoshi Kurihara, Professor and Director Junko Fujigasaki, Assistant Professor Takahiro Fukuda, Assistant Professor

General Summary

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

Research Activities

Histopathological safety evaluation of transcranial low-frequency ultrasound in the brains of stroke-prone spontaneous hypertensive rats

The main goal in the treatment of acute ischemic stroke is prompt arterial recanalization. Thrombolysis with recombintant tissue plasminogen activator (rt-PA) is efficient in humans but has significant problems, including slow and incomplete recanalization, frequent bleeding complications, and a limited therapeutic window. Therefore, adjunctive therapies are needed to extend the reperfusion time window, to increase efficacy, and to reduce the side effects of rt-PA. Ultrasound insonation has recently attracted attention as an adjunctive therapy. Low-frequency ultrasound (<1 MHz) is, however, believed to be unsafe, and high-frequency ultrasound (2 MHz) is largely ineffective. The major objective of this study was to examine the safety of low-frequency ultrasound thrombolysis in stroke-prone spontaneous hypertensive rats. Low-frequency ultrasound with a spatial-peak temporal-average intensity (Ispta) in the brain of 0.14 W/cm² at 297.3 kHz and 268.4 kHz showed an increased rate of cortical necrosis. With ultrasound with an Ispta in the brain of 0 to 0.06 W/cm² at 489.6 kHz, the percentage of cases with hsp70-immunoreactive cells and the number of hsp70-immunoreactive cells were increased in rats treated with ultrasound with a higher Ispta in the brain.

Ubiquitin — small ubiquitin-like modifier — positive neuronal intranuclear aggregates formed by proteasome inhibitor: Relation with intranuclear domains

Treatment with a proteasome inhibitor (MG-132) of neuronal cells (SH-SY5Y) produces ubiquitin-positive cytoplasmic and intranuclear aggregates. We have previously reported that the intranuclear ubiquitinpositive aggregates are SUMOylated and associated with promyelocytic leukemia protein (PML) — positive nuclear bodies. The ubiquitin — small ubiquitin-like modifier (SUMO) — positive aggregates have protein components similar to those of intranuclear inclusions observed in polyglutamine diseases or neuronal intranuclear inclusion disease. We examined the relation between the intranuclear ubiquitin-SUMO — positive aggregates and nuclear domains, including
PML-positive nuclear bodies, Cajal bodies, splicing factor compartments, and nucleoli. After treatment with high doses of MG-132, small dot-like and larger, irregular ubiquitin-SUMO — positive aggregates appeared. With lower doses, small, dot-like aggregates appeared, although larger aggregates were rare. The small aggregates were colocalized with PML-positive nuclear bodies and were occasionally adjacent to coilin-positive Cajal bodies, suggesting that the small aggregates were linked to the nuclear body — Cajal body domains related to a small nuclear RNA gene locus. The larger aggregates were surrounded by fibrillarin-positive structures, indicating that the aggregates might be associated with the nucleolus.

Publications

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Department of Neuroscience Laboratory of Neurophysiology

Fusao Kato, Professor and Director

General Summary

We are aiming to clarify the mechanisms underlying dynamic cell-to-cell signaling in the central nervous system. 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 for various types of disease, and animals with experimental manipulation of gene expression.

Research Activities

Regulation of synaptic transmission in the brain network

1. Molecular mechanism of neurotransmitter release

To clarify the roles played by specific molecules in transmitter release in brain synapses, we developed a novel method for *in-vivo* gene silencing with RNA interference against the genes encoding presynaptic proteins which is followed by functional analysis of synaptic transmission with patch-clamp recording in brain slices.

2. Glia-neuron interaction at synapses

We have demonstrated that pharmacological activation of astrocyte-specific P2Y1 receptors in brainstem slices facilitates glutamate release in a manner sensitive to P2X receptor blockade and gliotoxin (fluoroacetate) pretreatment. To our knowledge, this is the first study to demonstrate direct excitatory action of ATP of astrocyte origin on synaptic transmission.

3. Central mechanism of frequency-dependent decoding of afferent information

To understand how the brain analyzes sensory signals from internal organs, we analyzed the postsynaptic responses of second-order neurons in the nucleus of the solitary tract and the dorsal motor nucleus of the vagus nerve to repeated stimulation of afferent fibers. These synapses showed distinct types of short-term plasticity with distinct Ca^{2+} -dependency, which might underlie the frequency-dependent "tuning-in" of visceral information.

Central mechanisms of pain-related negative emotion

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

The role of monocarboxylate transport in the synaptic function

To clarify the role played by lactate uptake in synaptic transmission, we analyzed the effect of a selective inhibitor of monocarboxylate transporters on postsynaptic transmission in neurons of the nucleus of the solitary tract. We found that lactate transport is essential for maintaining postsynaptic responses mediated by alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors.

Molecular target of volatile anesthetics

We presented evidence that sevoflurane directly excites neurons in the locus coeruleus through an opening of gap-junction channels, a mechanism that might underlie the aberrant excitatory effect of this anesthetic frequently reported in clinical practice.

Publications

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Department of Genetic Disease Research (Lysosomal Storage Disease)

Yoshikatsu Eto, Professor

Research Activities

This Department was established on April 1, 2007 as a donated department. The members of this department are 1 principal investigator, 5 co-investigators, 1 visiting fellow, and 3 assistants collaborating with the Department of Gene Therapy, Institute for DNA Medicine.

The major topics for investigation are follows.

1. Development of screening methods for lysosomal storage diseases by means of dry blood spots.

The lysosomal storage diseases (LSDs) screened for were Pompe disease, Fabry disease, and mucopolysaccharidosis (MPS) I, II, and VI.

a) We screened at high risk patients with muscular dystrophy by means of dry blood spots (DBSs). About 120 patients from a muscular dystrophy hospital were analyzed for Pompe disease. Two patients showed markedly decreased activities, and gene analysis demonstrated that these patients had a pseudodeficiency of alpha-glucosidase.
b) Screening for Fabry disease in a dialysis center by means of DBSs. We screened more than 1,000 patients with end-stage renal disease.

c) Screening of patients for MPS I, II, and VI in a rehabilitation hospital by means of DBSs. We established a method of screening for MPS using DBSs. Our procedure used 4-methylumbelliferone as a substrate and 3-mm-diameter punched discs.

1) Gene therapy for Pompe disease, Fabry disease, and Twitcher disease using a lentivirus vector. Pompe knockout mice were successfully treated with a lentivirus vector, as shown by the markedly reduced levels of accumulated glycogen in various muscle tissues.

2) Isolation of induced pluripotent stem cells: We successfully produced induced pluripotent stem cells from tail-skin fibroblasts of Fabry, Pompe, and Twitcher mice. Induced pluripotent stem cells from Fabry mice were differentiated into cardiac muscle cells.

Publications

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Institute for High Dimensional Medical Imaging

Naoki Suzuki, Professor and Director

Asaki Hattori, Assistant 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

1. Clinical application of high dimensional medical imaging with virtual reality techniques

We are developing technology for high dimensional medical imaging using biological and morphological data sets from X-ray computed tomography (CT) and magnetic resonance imaging (MRI). In our research we study the visualization of 4D-CT data sets and develop clinically applicable methods of 3D and 4D image data management and visualization. This research is performed in collaboration with such institutions as Kyushu University, Osaka University, Tsurumi University, and the Mayo Clinic (Rochester, MN, USA).

2. Surgery navigation system development

We are developing a navigation system that can overlay the 3D images of the internal structure of the body onto the surgical field. We have developed navigation systems for robotic surgery systems and have designed and built a high-tech navigation operating room at Daisan Hospital. This year, we performed 2 navigation surgeries in this operating room. In joint research with the department of otorhinolaryngology, we examined the use of our navigation system for microscope surgery and stereoendoscopic surgery.

3. Endoscopic robot surgery system development

We are developing an endoscopic robot surgery system that can be used to perform natural orifice transluminal endoscopic surgery, which is a technique for operating on abdominal organ via the mouth. We have developed endoscopic surgery robots that enable the surgeon to more freely perform surgical manipulations in the gastrointestinal tract using a pair of forceps-type manipulators. This year, we developed a robotic telesurgery system that allows a surgeon to perform an operation from a remote location by controlling a robot via a computer network. After a preliminary experiment, we performed a robotic telesurgery experiment with a soft cadaver via a high-speed computer network between Japan and Thailand. In the experiment, a robot that we controlled from Japan was used to excise a lymph node from the abdominal cavity of soft cadaver with a digestive tract cancer in Thailand. We are collaborating with Kyushu University on this project.

4. Surgery simulation system development for robotic surgery

An operation using the robotic surgery system described above is very different from a normal operation and requires advanced training to perform. Therefore, we are developing a surgical simulation system that allows the same surgical procedure to be performed in a virtual space with an internal organ model constructed from X-ray CT and MRI data sets of a patient. This year we constructed a soft-tissue model of a channel that reaches the stomach for training. Using a force-feedback device, the surgeon operates a forceps-shaped robot arm in virtual space and can change the shape of the gastric wall in real time.

5. Development of a visualization and analysis system for temporomandibular joint activity

We are developing a 4D system for real-time quantitative analysis of the movements of the lower jaw. First, we reconstructed a 3D skeletal model using X-ray CT data of the upper and lower jaws of a patient with a temporomandibular joint disorder. Second, we activated the skeletal model using data of the patient's jaw movements. This year we performed an analysis in virtual space, created a plaster model, activated it with a multiple-joint robot, and were able to analyze the jaw movements in real space. By analyzing both the virtual and real spaces, we could exploit the advantages of both spaces and could more effectively establish diagnoses and create treatment plans. We are performing this research project in collaboration with Tsurumi University.

Publications

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Reviews

Hattori A, Suzuki N, Suzuki S, Otake Y. Development of an endocsopic robot system for NOTES (in Japanese). *Nihon Computer Geka Gakkaishi* 2007; **9:** 79–84.

Institute of Clinical Medicine and Research

Kiyotaka Fujise, Professor and Director Akihito Tsubota, Associate Professor Yoshihisa Namiki, Assistant Professor Sadayori Hoshina, Associate Professor Koichi Nariai, Assistant Professor

General Summary

The Institute of Clinical Medicine and Research always conducts research with a focus on clinical applications. In 2007, the institute performed medical research related to the etiology, diagnosis, and treatment of intractable diseases. As part of this research, we examined the relationship between oxidative stress and disease, including the effect of oxidative stress on hepatocarcinogenesis, the role of reactive oxygen species (ROS) in physiological and pathological conditions, and the usefulness of antioxidant agents. Additionally, we have performed studies to define the pathological conditions underlying hyperlipidemia and heart disease, to clarify the action of anesthetics against substances associated with pain, to prepare nanoparticles for poorly soluble drugs using innovative procedures, and to define the pathological conditions underlying viral diseases.

Research Activities

Liver disease and oxidative stress

1. Comprehensive RNA gene expression analysis of hepatocarcinogenesis in oxidative stress-induced chronic liver damage: Our group investigated how oxidative stress conditions influence oncogenesis in chronic liver damage, using an animal model of persistent exposure to ROS and comprehensive RNA gene expression analyses.

2. Development of a novel antioxidant agent: The effect of bovine lactoferrin on oxidative stress-induced chronic liver damage was investigated in an animal model. We have clarified the mechanism of lactoferrin action and have developed a novel drug delivery system, including intestine-dissolved and PEGylated types. An international patent application for this system has been submitted.

3. Analysis of the pathogenesis and development of treatment in chronic hepatitis C virus infection: We are aiming to improve the efficacy of antiviral treatment for chronic hepatitis C virus infection by analyzing viral kinetics and applying clinical protocols, including randomized controlled trials, in collaboration with the Division of Gastroenterology and Hepatology, Kashiwa Hospital.

Ovulation inhibition due to removal of peripheral blood phagocytes

ROS containing superoxide are involved in ovulation. We have recently used a specific superoxide sensor to confirm production of superoxide and have used immunohistochemical studies to localize DNA and lipid peroxides in the ovulating ovary. Phagocytes, such as neutrophils and macrophages, are thought to be sources of ROS involved in ovulation. This year, we have started to examine whether removal of peripheral blood phagocytes inhibits ovulation, to examine the source of ROS involved in ovulation.

The development of a magnetic drug delivery system for cancer

The desired performance of cancer treatment has not been achieved with conventional drug delivery systems. To overcome this problem, since 2006 we have developed novel magnetic nanoparticles that include anticancer agents. Moreover, we have developed strong magnets to accumulate these nanoparticles at tumor lesions. This work has been supported by Industrial Technology Research Grant Program 08C46049a in 2008 from New Energy and Industrial Technology Development Organization (NEDO) of Japan, the Takeda Science Foundation in 2007 and the Tsuchiya Foundation in 2006.

The development of molecular prevention of metastasis of colon cancer

The hepatocyte growth factor analogue NK-4 strongly inhibits angiogenesis and tumor metastasis. To prevent colon cancer from metastasizing to the liver, since 2006 we have investigated the effect of the oligomannose-coated lipid nanoparticles that contain the NK-4 gene.

Clinical microbiology

DNA diagnosis of clinical mycosis was established with the mycotic universal sequence and applied to infectious tissues. The minimum inhibitory concentration of a *Pseudomonas aevuginosa* was measured with an oxygen potential electrode system, which was applied clinically.

The dioxin chemical structure was proposed for anti-inflammatory, antiproliferative agents from the aspect of the resistance mechanism of the themophilic bacterium *Bacillus midousuji*.

Publications

Koyama T, Tsubota A, Nariai K, Mitsunaga M, Yanaga K, Takahashi H. Novel biomedical imaging approach for detection of sentinel nodes in an experimental model of gastric cancer. Br J Surg 2007; 94: 996-1001.

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

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Yanai H, Yoshida H, Hirowatari Y, Tomono Y, Tada N. Oxidized low density lipoprotein elevates platelet serotonin release. Am J Hematol 2007; **82:** 686-7.

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Medical Engineering Laboratory

Hiroshi Furuhata, Professor and Director

Kouichi Kanemoto, Associate Professor

General Summary

The Medical Engineering Laboratory has developed new ultrasound (US) therapeutic technologies. This year, with the support of a research grant from the Ministry of Health, Labour and Welfare, we focused on the development of new thrombolytic treatments for patients with acute ischemic stroke. Basic research studies in molecular medical engineering have also been continued to develop ultrasonic drug delivery system. In particular, the safety of phase-change nanodroplets from fluid to gas by ultrasonication has been evaluated histopathologically, and the increase in nitric oxide (NO) generation by ultrasonication has been analyzed by means of immunochemical real-time monitoring. We have contributed to the development of a national database on less-invasive medical devices that includes information on less-invasive technology from around the world and gathers the needs of clinicians. We have also improved previous-ly developed diagnostic techniques for measuring cerebral circulation and hemodynamic variables by the means of noninvasive transcranial ultrasonography.

Research Activities

An integrated US system for the diagnosis, analysis, and treatment of acute stroke This system uses transcranial ultrasonication to enhance the thrombolytic effects of recombinant tissue plasminogen activator (rt-PA) in the treatment of acute ischemic stroke according to navigation with US performed with a single probe. For the clinical application of this system, we have developed a new device for holding the US probe near the patient's head. Because many Japanese patients have an insufficient temporal bone window, we developed a brain virtual ultrasonography method for the use of this sonothrombolysis system in these patients. This new technology can display as a US image in real time the same cross-sectional image obtained with magnetic resonance imaging or computed tomography.

Ultrasonic thrombolysis

The accelerating effect on thrombolysis of combined use of low-frequency (500-kHz) US, rt-PA, and bubble liposomes was verified *in vitro*. Bubble liposomes have a great potential for accelerating the thrombolytic effect of rt-PA with continuous-wave US.

Verification of the safety of an ultrasonic drug delivery system for cancer therapy We have been developing an ultrasonic drug delivery system that is integrated with an ultrasonic diagnostic and therapeutic system with phase-change nanodroplets to provide US images of tumors and to simultaneously kill tumor cells with US heating effects for the selective treatment of tumors. We established a method to verify damage to normal tissue surrounding tumors by means of histopathological evaluation with hematoxyline/ eosin and Masson's stain.

NO generation by US stimulation

We have reported that the NO concentration in the thigh adductor muscle of rabbits increased with the intensity of applied US. Moreover, we have also demonstrated that US stimulation enhances NO generation in tumors.

Development of database for less-invasive medical devices

On the basis of an existing database of nanomedicine, we have developed a new database to provide a "knowledge infrastructure for minimally invasive medical technology." This database can be accessed via the Internet and includes a special forum for discussions of various less-invasive technologies. This development was supported by a research grant from the Ministry of Health, Labour and Welfare.

Publications

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Division of Clinical Pharmacology and Therapeutics

Shigeru Kageyama, Professor and Director

General Summary

The Division of Clinical Pharmacology and Therapeutics was established in July 1995. The aim of the division is to investigate drug treatment, mainly in the area of internal medicine, whereas other departments of clinical pharmacology in Japan focus on registration trials, particularly phase I trials. Because a clinical laboratory where we had performed many human pharmacological studies became unavailable in 2003, we shifted our research from human studies to multicenter clinical trials and pharmacoe-pidemiological studies.

Research Activities

We have performed a pharmacoepidemiological study of the prescription of antihypertensive drugs in patients with diabetes. Patients with hypertension complicated by diabetes are considered to be at high risk for cardiovascular events; therefore, the target blood pressure is set lower than for patients who have hypertension but no diabetes and the drugs of choice are different. We investigated the use of antihypertensive drugs in patients who have hypertension with or without diabetes by means of prescription data from 3 university hospitals.

Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) have been widely used to treat hyperlipidemia. They have adverse effects on muscle, the liver, kidneys, and other organs. To investigate the incidence of these adverse effects and antihyperlipidemic effects, we started a pilot study in 3 major hospitals, including our hospital, according to a case-cohort study design in which detailed data were collected in all cases and in a subcohort representing 5% of the whole sample. A full-scale study will be conducted with a large sample size of 20,000 to 30,000 patients.

An important issue for medicine in the 21st century is to identify patients who are responsive or unresponsive and those who show or do not show adverse reactions to drugs. We started a collaborative study with other institutions to examine the relationship between drug-metabolizing enzyme gene polymorphisms and drug effects in residents of an isolated island. We have analyzed the drug-metabolizing enzymes CYP2C9 and CYP2C19. Some of the results have already been applied to drug therapy.

An administrative office for registration trials was established in the hospital in February 1999, and the system for registration trials in the hospital has been reformed to meet the demands of the new good clinical practice guidelines. Seven clinical research coordinators (CRCs) — 6 nurses and 1 pharmacist — now facilitate clinical trials. The CRCs have started to help with both registration trials and investigator-initiated trials. CRCs were introduced into all registration trials since 2004; the quality and speed of these trials were much improved.

The Ministry of Health, Labour and Welfare started a New 5 Yearly Clinical Trial Action Plan to help registration trials to cope with trials done abroad. This action plan selects 10 core hospitals and 30 major clinical trial institutions. The Jikei University Hospital applied to be a major clinical trial institution and was accepted. According to this plan, we reinforced CRCs and introduced data managers to improve the clinical trial system.

Publications

Saito T, Tojo K, Nishimura R, Kageyama S, Tajima N. Coefficient of variation of R-R intervals in electrocardiogram is a sensitive marker of anemia induced by autonomic neuropathy in type 1 diabetes. Diabetes Res Clin Prac 2007; **78**: 60–4.

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Kageyama S. α blocker (in Japanese). In: Kumagai H, et al. editors. Koketsuatsu Nabigehta. 2^{nd} ed. Tokyo: Medical View; 2008. p. 248-9. **Yoshida H, Kageyama S.** Actions and effects of cannabinoid receptor antagonists (in Japanese). *In: Kanazawa Y, et al.* editors. Annual Review Tonyobyo Taisya Naibunpitsu. Tokyo: Chugaiigakusya; 2008. p. 104-11.

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Sugiyama Y, Mayahara H, Ikeda T, Yano T, Itoh K, Suhara T, Kurihara C, Unno T, Sagami F, Otsuka M, Kato M, Tsuji A, Miura S, Inoue T, Kawakami K, Zanka A, Hiyama Y, Suzuki K, Yanai K, Tozuka Z, Nishimura S, Watanabe Y, Kageyama S, Kumagai Y, Fujiwara H, Watanabe H. Basis for the conduct of microdosing clinical trials in Japan: Third report draft guidance for exploratory investigational new drug clinical trials (in Japanese). Rinsho Hyoka 2007; **34**: 571-94.

DDS Institute

Megumu Higaki, Professor and Director Naomi Yamashita, Professor Yutaka Mizushima, Professor Tsutomu Ishihara, Assistant Professor

General Summary

We are investigating a new drug delivery system (DDS) using nanotechnology. We have developed polylactic acid (PLA)/polyethylene glycol (PEG)-PLA nanoparticles for targeting and sustained release of steroid/immunosuppressants and have demonstrated enhanced anti-inflammatory activity in experimental animal models of arthritis. These studies were supported in part by a grant from the Ministry of Health, Labour and Welfare of Japan. We also prepared thermosensitve nanoparticles using N-isopropylacrylamide (NIPAAm) to control cellular uptake. The presence of CD208-positive keratinocytes was shown in psoriatic epidermis.

Research Activities

Nanoparticle preparations of a steroid for targeting and sustained release

The purpose of this study was to engineer nanoparticles with various sustained profiles of drug release and prolonged circulation by blending PLA/poly(D, L-lactic/glycolic acid) (PLGA) homopolymers and PEG-block-PLA/PLGA copolymers encapsulating betamethasone phosphate (BP). Nanoparticles of different sizes, drug encapsulation/ release profiles, and cellular uptake levels were obtained by mixing homopolymers and block copolymers with different compositions/molecular weights at various blend ratios with an oil-in-water solvent diffusion method. The in vitro release of BP increased with smaller nanoparticles or PLGA homopolymers instead of PLA homopolymers. Furthermore, the uptake of nanoparticles by macrophage-like cells decreased with nanoparticles of higher PEG content, and nanoparticles of PEG-PLGA block copolymers were taken up earlier than were nanoparticles of PEG-PLA block copolymers after incubation with serum. In addition, prolonged blood circulation was observed with nanoparticles of smaller size with higher PEG content, and nanoparticles of PEG-PLA block copolymers remained longer in circulation than those of PEG-PLGA block copolymers. Analysis of BP concentration in organs and in vivo fluorescence imaging revealed that the liver distribution of blended nanoparticles was less than that of PLA nanoparticles. To our knowledge, this is the first study to systematically design and characterize biodegradable PLA/PLGA and PEG-PLA/PLGA-blended nanoparticles encapsulating BP with different release profiles and stealthiness.

The PLA nanoparticle preparations (about 150 nm in a diameter) containing BP with zinc ion was confirmed to be an adequate DDS because of the absence of an initial burst. The pharmacological effect of a single intravenous injection of this preparation continued for 1 week in several experimental animal models of inflammation. The pharmacological potency of this preparation was 2 to 4 times greater than that of

betamethasone sodium phosphate.

Because this preparation showed significant accumulation in the reticuloendothelial systems of the spleen and liver, PEGylation of the nanoparticles was performed with PEG-PLA block polymers. Because stealth nanoparticles escaped trapping in the liver and specifically accumulated in the inflammatory lesions, they showed an antiinflammatory effect 5 to 10 times greater than that of nonstealth nanosteroids in animal models and had reduced adverse effects. Production at an industrial scale according to the guidelines of good manufacturing practices of active pharmaceutical ingredients is also under investigation.

Publications

Ishihara T, Takahashi M, Higaki M, Takenaga M, Mizushima T, Mizushima Y. Prolonging the in vivo residence time of prostaglandin E1 with biodegradable nanoparticles. *Pharm Res* 2008; **25:** 1686–95.

Division of Clinical Research and Development

Satoshi Kurihara, Professor Masato Matsushima, Associate Professor Mitsuyoshi Urashima, Associate Professor and Director

General Summary

Clinical study is a strategy and a science for implementing 1) efficient diagnosis, treatment, and prevention, 2) the discovery of etiology, and 3) understanding pathophysiology. Baron Kanehiro Takaki prevented beriberi by a change in diet based on evidence obtained from clinical study. Thus, we at The Jikei University School of Medicine have a responsibility to carry on and expand his passion using epidemiology and biostatistics to save lives and maintain peace in the world. However, most clinicians are busy treating patients. In addition, modern epidemiology and biostatistics have been changing along with computer science. Therefore, we have established the Division of Clinical Research and Development as a project base to support clinical studies at The Jikei University School of Medicine. Our mission is to find *veritas* in a complicated clinical world and to contribute to society with our findings. To accomplish our mission, education and promotion are major strategies.

Research Activities

The Jikei clinical research course

From May 2007 through March 2008, we held 21 seminars about strategies for clinical studies for health-care practitioners at The Jikei University. In 2007, small-group study courses targeting postgraduate students will be started from the principles of epidemiology and biostatistics by reading textbooks and by analyzing real clinical data using STATA software and designing clinical studies. Our goal is for postgraduate students to develop the skills to construct hypotheses, design protocols, monitor trials, and analyze data.

Consulting for clinical studies

- 1. Inside The Jikei University: Most clinical departments
- 2. Outside The Jikei University: Tokyo Women's Medical University

As shown in the publication section, we published several articles in English by collaborating with clinical departments. For fiscal year 2007 we accelerated the process from consultation to paper submission. Next year, we are planning to launch a clinical data-pooling system in our division to support high-quality prospective clinical studies.

Umbilical cord blood study

We hypothesized that exposure of fetuses to low concentrations of hazardous heavy metals during pregnancy can cause changes in the behavior and intelligence of children. We have started to collect umbilical cord blood to measure heavy metals and have sent questionnaireS to ask about daily habits and the condition of children after birth. We have finished enrollment, since we could collect more than 1,000 samples of umbilical cord blood. We have finished measuring levels of heavy metals in umbilical cord blood and collected questionnaires from when the children were 2 years old. In addition, we are performing a study of twins to examine the genetic and environmental effects on children's behavior.

Molecular epidemiology

Genes of epidermal growth factor beta in cancer cells obtained from clinical specimens were sequenced, and several mutations of the epidermal growth factor receptor were confirmed to generate tumors in vitro. Moreover, we found that the combination of mutation and phosphorylation might be a prognostic indicator.

Diagnosis of complication in diabetes mellitus

We could draw receiver operating characteristics curves with high sensitivity and high specificity for diagnosing complications of diabetes mellitus.

Global environmental change and human health

We have been dedicated to research on global environmental change and human health with the Japanese government.

Homeland security

Preventing acts of terrorism is one of our missions. An extreme example of prevention may be homeland security. We attended a workshop on "G8 Forensic Epidemiology" in London. We have held a series of lectures on national security affairs and crisis management for the Cabinet and have advised the Deputy Chief Cabinet Secretary for Crisis Management.

To assess communication capacity between remote locations, governments, ministries, and the cabinet under extraordinarily stressful conditions during national crises, the Biosecurity 2007 tabletop exercise was held in October 2007 at The Jikei University School of Medicine. It was the largest biosecurity exercise of its kind in Japan. The scenario was that an ecoterrorist group had released the severe acute respiratory syndrome virus, a CDC category C agent, as well as anthrax, a CDC category A agent, at different sites on the same day as an overt act of bioterrorism.

Publications

Ueda K, Yamada K, Urashima M, Ishibashi Y, Shirai M, Nikaido T, Takahashi H, Okamoto A, Saito M, Yasuda M, Ohkawa K, Tanaka T. Association of extracellular matrix metalloproteinase inducer in endometrial carcinoma with patient outcomes and clinicopathogenesis using monoclonal antibody 12C3. Oncol Rep 2007; **17:** 731-5.

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Laboratory Animal Facilities

Kiyoshi Ohkawa, Professor and Director Koichi Nariai, Assistant Professor Takamasa Iwaki, Associate 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 2007, 186 researchers used the LAF. We undertake breeding of experimental animals and provide technical guidance to researchers performing animal experiments. Furthermore, we performed the following studies to develop basic medical sciences, including laboratory animal science.

Research Activities

Publishing anatomic color atlases of laboratory animals

Anatomical information on laboratory animals is necessary for animal experimentation. We have already published 3 anatomical atlases of laboratory animals (rabbit in 1993, rat in 1997, and mouse in 2001) in collaboration with the Department of Anatomy (I). We are preparing to publish international editions of these atlases to spread the information throughout the world.

Establishment and characterization of strains derived from Japanese wild mice and Phodopus hamster

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

Japanese wild mice are excellent sources of genetic material to improve laboratory mice, because the genetic constitution of this subspecies differs greatly different from that of common laboratory mice derived from subspecies. We have established several new inbred strains based on Japanese wild mice captured in Osaka prefecture. These strains are being maintained in our laboratory, and new consomic strains based on these strains are being developed.

Phodopus hamsters are small rodents that differ taxonomically from Syrian hamsters, which are the major laboratory hamster. We recently confirmed that the *Phodopus* hamster is a promising candidate for a new laboratory animal, and we have established an inbred strain. Furthermore, we continue to establish other inbred strains or congenic strains, to develop human disease models, and to investigate their biomedical characteristics.

The search for a novel drug for treating atopic dermatitis by means of an NC/Nga inbred mouse strain

The NC/Nga inbred strain is the current mouse model for atopic dermatitis. However,

the rates of dermatitis differ among laboratories. The NC/Nga inbred strain maintained in our laboratory has a diathesis for particularly severe dermatitis. In collaboration with the Department of Tropical Medicine, we are using NC/Nga mice to search for novel drugs for treating atopic dermatitis.

Development of a simple method of tracheal intubation for small experimental rodents

Airway management is an important technique related to "refinement" and "reduction" of the "3 R's" of animal experimentation. Tracheotomy is a classic technique in rodents but causes severe stress. An otoscope is used as a laryngoscope for tracheal intubation. Tracheal intubation guided by fiberoptic endoscopy is also presented. For these methods, particular equipment is required, and there are matters of safety and reproducibility. To solve these problems, we developed, in collaboration with Department of Internal Medicine (Cardiology), a simple method of tracheal intubation for small experimental rodents.

Ovulation inhibition due to removal of peripheral blood phagocytes

Reactive oxygen species containing superoxide are believed to be involved in ovulation. We have recently confirmed, by means of a specific superoxide sensor, production of superoxide and found immunohistochemical localization of DNA and lipid peroxides in the ovulating ovary. Phagocytes, such as neutrophils and macrophages, are thought to be sources of reactive oxygen species involved in ovulation. This year, we started to examine whether the removal of peripheral blood phagocytes inhibits ovulation, to examine sources of reactive oxygen species involved in ovulation.

Publications

Watanabe N, Tomimori Y, Terakawa M, Ishiwata K, Wada A, Muto T, Tanaka T, Maruoka H, Nagahira K, Nakatsuka T, Fukuda Y. Oral administration of chymase inhibitor improves dermatitis in NC/Nga mice. J Invest Dermatol 2007; 127: 971–3.

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Radioisotope Research Facility

Kunihiko Fukuda, Professor and Director

Yukio Yoshizawa, Assistant Professor

General Summary

The Radioisotope Research Facility was established to support medical and biological research with isotopes. We have supported researchers by suggesting methods and practical techniques for experiments. Lectures and training are held for researchers and for medical students and graduate students who are starting to work with radioisotopes. In 2007, 66 researchers in 15 departments consulted this facility for 29 studies. On March 19, the facility passed an inspection by the Nuclear Safety Technology Center. This inspection confirmed that the facility is maintained in compliance with technical standards and that the radiation control was appropriately performed.

Research Activities

Isolation of fibronectin binding protein — deficient mutants of Staphylococcus aureus Staphylococcus aureus has a variety of adhesins on its cell surface. Among these proteins, fibronectin binding proteins (FnBPs) are crucial for interacting with host cells. We isolated FnBP A-deficient mutants by inserting a tetracycline-resistance gene into the *fnb A* gene. The mutant strain and the parental SH1000 strain were used to infect mice, and the virulence of the strains was determined. The results indicate the importance of FnBP A for invading host tissues. To determine the role of FnBP B in *in vivo* infection, we are attempting to isolate *fnb B* gene mutants.

The synergistic activity of glycopeptides with beta-lactams against methicillin-resistant S. aureus

Glycopeptide antibiotics inhibit peptidoglycan synthesis by binding to the terminal D-Ala-D-Ala stem of peptidoglycan precursors. Although a synergistic effect is expected when the glycopeptides are combined with beta-lactams, which inhibit cell wall synthetic enzymes, 3 kinds of results — synergistic, no change, and antagonistic — are obtained. Cefpirome and ceftizoxime, which show antagonistics effects against vancomycin, have low affinity for penicillin binding protein (PBP) 3 and PBP4, whereas imipenem and panipenem, which show synergistic effects, have high affinity for all 4 PBPs. The ability of the beta-lactams to inhibit PBP1 — PBP4 is essential for the synergistic effect.

Analysis of the mechanism of resistance in radiation-resistant organisms

Tardigrades (water bears) are highly resistant to radiation, but the mechanism of resistance remains unclear. Therefore, we have isolated tardigrades from mosses grown on the streets around The Jikei University and examined their resistance to dehydration, temperature, and radiation of the tardigrades of the family *Macrobiotidae* and *Milnesium*

tardigradum, identified on the basis of morphologic features.

A study of radioactivity in consumer products

We have measured gamma rays emitted from deodorants that are said to have "minusion" effects. The results revealed that these products contain the daughter nuclides of the Th and U series as well as ⁴⁰K. Three deodorants were likely to be radioactive consumer products through the intentional addition of radioactivity. One cupboard deodorant had a relatively high whole-body dosage of 0.17 μ Sv•h-1 at the distance of 1 m.

Radon gas is the most important risk factor for lung cancer. The ²²²Rn concentrations emanating from the "minus-ion" wallpapers were measured using Pico-Rad radon detectors (AccuStar Labs, Medway, MA, USA). The maximum radon concentration was 34 Bq·m⁻³, which was twice the average indoor value of 15.6 Bq·m⁻³. We believe that wallpaper that emits radioactivity can unnecessarily increase indoor radon exposure.

Research on marine bacteria

The habitat distribution of several marine bacteria was surveyed during *Tansei Maru* cruise KT-07-16. To study the metallic transport system of microbes, we focused on marine bacteria that produce siderophores in a low-iron environment. We are attempting to cultivate such bacteria from seawater and the sediment of the sea floor at a depth of 3,000 m.

Publications

Minowa H, Takeda M¹, Ebihara M¹ ('Tokyo Metroplitan Univ). Sequential determination of ultra-trace highly siderophile elements and rare earth elements by radiochemical neutron activation analysis: application to pallasite meteorites. J Radioanal Nuclear Chem 2007; 272: 321-5. Furuta E, Yokota S, Aburai T, Yoshizawa Y. Evaluation of radiation exposure from shoedeodorants as radioactive consumer products (in Japanese). *RADIOISOTOPES*. 2007; **56**: 443-53.

Furuta E, Moriata-Murase Y, Yoshizawa Y. Evaluation of internal/external exposure from interior building materials (in Japanese). *Hoken-buturi* 2007; **42:** 341-8.

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

Running training increases heat shock proteins in skeletal muscle

Heat shock proteins (HSPs) play important roles in protecting cells from stress by acting as molecular chaperons. Limited information is available about the responses of HSPs to training and whether the frequency of training affects changes in HSPs. We investigated the effects of training frequency on the expression of several HSPs in the gastrocnemius muscle of rats. The trained rats ran (30 m/minute) on a motor-driven treadmill for 3 days per week (T3 group) or 6 days per week (T6 group) for 6 weeks. The total running duration was 60 minutes for the T3 group and 30 minutes for the T6 The expression levels of HSPs of 73, 72, 60, 40, and 25 kD and of α B crystallin group. were determined in the white and red regions of the lateral gastrocnemius muscles using the Western blotting method. No significant increase in expression of any of the HSPs was evident in the red region after training in either the T3 or T6 group. In contrast, the expression levels of all HSPs except for HSP73 and HSP60 increased in the white region after training in both groups without a difference between the groups. The lack of significant difference between the T3 and T6 group suggests that the total training load rather than the training frequency contributes to the increases in HSPs. We conclude that the increases in HSP expression after endurance training are region-specific and are not affected by training frequency.

Relationship between eating and hoarding behavior and neuropeptide Y mRNA in the arcuate nucleus of the hypothalamus of exercising Syrian golden hamsters

Syrian golden hamsters more actively perform wheel-running exercise than do rats and have a strictly defined circadian rhythm for running activity. For these reasons, we hypothesized that the factors regulating appetite behavior would be changed in exercising Syrian golden hamsters compared with those in ad libitum controls. Moreover, Syrian golden hamsters have a hoarding habit, which reflects their long-term appetite. Therefore, we used Syrian golden hamsters to evaluate the relationship between eating and hoarding behaviors and neuropeptide Y mRNA in the arcuate nucleus during wheel running exercise. We found that the low-appetite condition was indicated by decreases in the eating and hoarding volumes of food in exercised hamsters. Serum leptin levels were markedly lower in exercising hamsters than in ad libitum controls. No significant

difference was found in leptin receptor mRNA levels in the arcuate nucleus of the hypothalamus. Also, as we had hypothesized, neuropeptide Y mRNA levels in the arcuate nucleus of the hypothalamus were significantly lower in exercising hamsters than in ad libitum controls. Therefore, we speculate that a mechanism of exercise-induced appetite inhibition is a decrease in neuropeptide Y in the arcuate nucleus of the hypothalamus.

Exercise intensity affects plasma concentrations of tumor necrosis factor alpha in response to lipopolysaccharide in rats

Moderate exercise can reduce the risk of infectious disease. In contrast, strenuous exercise transiently increases the risk of infection. We examined the mechanisms underlying these observations. Ten-week-old female F344 rats were randomly assigned to 1 of 4 groups: rest (R) and low (LE)-, middle (ME)-, and high (HE)-intensity running exercise. Immediately after the exercise or resting period, rats were given intravenous injections of lipopolysaccharide (LPS) (1 mg/kg). Blood samples were obtained immediately after exercise or rest and 1 hour after the LPS injection. Plasma concentrations of tumor necrosis factor (TNF)-alpha in both the ME and HE groups were significantly lower than those in the R and LE groups, respectively. Exercise intensity did not affect plasma corticosterone concentrations. In contrast, plasma epinephrine concentrations in the ME and HE groups were significantly higher than those in R and LE groups, respectively. In addition, plasma norepinephrine concentrations in the HE group were higher than those in the other 3 groups. Responses of TNF-alpha to LPS were suppressed in the ME and HE groups but not in the LE group. This immunosuppression might be regulated by changing catecholamine concentrations, which depend on exercise intensity.

Department of Cell Physiology Division of Aerospace Medicine

Satoshi Kurihara, Professor and Director Hiroko Toshima, Assistant Professor Masamichi Sudoh, Associate Professor

General Summary

Our main research interests are gravitational physiology, aerospace medicine, and stress reaction.

Research Activities

Optokinetic nystagmus and afternystagmus during the 7 days' bed rest

We analyzed the time-dependant change of optokinetic nystagmus (OKN) and optokinetic afternystagmus (OKAN), during a 7-day experiment of 6-degree head-down bed rest in which various biomedical variables were measured during long-term alteration of the direction of gravity. At the Tsukuba Space Center of the National Space Development Agency of Japan (now the Japan Aerospace Exploration Agency), 6 healthy men were studied. We collected OKN and OKAN data (both right and left directions) 6 times: in the sitting position on the day preceding bed rest, in the supine position on the 1st, 3rd, and 5th days of bed rest, and in the sitting position again immediately after bed rest on the 7th day and on the day following bed rest. The increase in OKN (OKN slow-phase-velocity/stimulus speed) tended to decrease on the 1st day of bed rest, had recovered gradually by the 5th day, and after bed rest exceeded the level before bed rest. The gain was higher when the OKN slow phase was directed rightward than when it was directed leftward throughout the 6 times. The appearance rate of the OKAN first-phase decreased during bed rest and recovered after bed rest to the level before bed rest. In contrast, the appearance rate of the OKAN second-phase increased on the 1st day of bed rest, and the increase was maintained thereafter, suggesting that an OKAN generator might differ between the first and second phases. The maximum slow-phase velocity of OKAN decreased markedly on the 1st day, but the recovery was unclear during and after bed rest. No clear tendency was found in the time-dependency of the OKAN duration. We speculate that these time-dependent changes observed in the optokinetic -oculomotor system are based mainly on the alternation in gravity information to the otolith organ and that bed rest is useful for studying long-term adaptation in the vestibular system under microgravity.

Stress

1. The processing of stress in the human brain and the stress-related disorders We performed near-infrared optical topography and recorded event-related P300 potentials when subjects felt stress. We then investigated information processing in the brains of human beings. 2. Brain imaging in *karuta* players by means of near-infrared optical topography and measurement of event-related potentials

We recorded the event-related P300 potentials and performed near-infrared optical topography of *karuta* players during an auditory oddball task that is similar to *karuta*, and we investigated information processing in the brains of *karuta* players.

3. Stress facilitates spontaneous platelet aggregation in healthy young persons After arithmetic stress loading, the concentration of norepinephrine was significantly increased. Spontaneous platelet aggregation was also accelerated by arithmetic stress, which was correlated with the increase in norepinephrine concentration. Stress releases norepinephrine from sympathetic nerve terminals, and the binding of norepinephrine to alpha-2 adrenoreceptors of platelets triggers platelet aggregation.

Publications

Nomura Y¹, Igarashi M¹, Sudoh M, Hida K¹, Sekiguchi C, Ishii M, Matsusima M, Kaneita Y¹, Ikeda M¹ (¹Nihon Univ). Optokinetic nystagmus and afternystagmus during the 7days bed rest study. (in Japanese). *J J Aerospace Environ Med* 2008; **45:** 3-9.

Department of Orthopaedic Surgery Division of Sports Medicine

Minoru Shiraishi, Assistant Professor

General Summary

Since the Division of Sports Medicine was established as the Sports Clinic in October 1985, we have performed research on sports medicine in the following subjects: 1. elite amateur and professional athletes; 2. middle-aged and elderly people who regularly do sports; 3. children and adolescents who participate in sports at school or in clubs; and 4. other areas of general sports medicine.

Research Activities

1. A series of research studies on hyperthermia were done. Results of general research into hyperthermia therapy for sport injuries of muscles and tendons were reported. The response of human skeletal muscle heat-shock protein 72 to microwave therapy was examined. The difference in the therapeutic effect of microwave therapy between 434 MHz and 2,450 MHz was studied.

2. We reported on medical support for a road racing team. Domestic and international stage races were examined. We reported on the medical support concerning advance preparations and emergencies during one season. The methods and problems of doping control were also examined.

3. Research regarding football was continued. The main aspects of the medical environment and medical support for an amateur football club that aims to become professional were reported. We discussed how to achieve an earlier return to action for athletes for whom the outcome of rehabilitation was poor.

4. We reported on a case of 1st rib stress fracture in a competitive dancer and examined the characteristics of dance injuries.

5. We reported on the treatment of shoulder muscle injuries.

Publications

Ichinoseki-Sekine N¹, Naito H¹, Saga N¹, Ogura Y¹, Shiraishi M, Giombini A (Italian Natl Olympic Committee), Giovannini V (Restek Med Device), Katamoto S¹ (¹Juntendo Univ). Effects of microwave hyperthermia at two different frequencies (434 and 2450 MHz) on human muscle temperature. J Sports Sci Med 2008; 7: 191–3. Sekine N¹, Naito H¹, Ogura Y¹, Shiraishi M, Sugiura T (Yamaguchi Univ), Katamono S¹ (¹Juntendo Univ). Changes in human muscle HSP72 induces by 434 MHz microwave hyperthermia (in Japanese). Tairyoku Kagaku 2007;

55: 602.

Shiraishi M, Ushijima F. Medical support in a football club aiming professional (in Japanese). *Tairyoku Kagaku* 2007: 56: 840. Ushijima F, Shiraishi M, Sato M, Nakajima Y, Nakamura Y. Medical support for the road racing team in an overseas stage race (in

Japanese). Tairyoku Kagaku 2007: 56: 849.

Review and Books

Giombini A (Inst Sport Med and Sci), Giovannini

V (Rome Univ), Di Cesare A, Pacetti P (Tor Vergata Univ), Ichinoseki-Sekine N¹, Shiraishi M, Naito H¹ (¹Juntendo Univ), Muffulli (Keele Univ). Hyperthermia induced by microwave diathermy in the management of muscle and tendon injuries. Br Med Bull 2007: **83:** 379-96. **Ide J¹, Ushijima F, Mizuta H¹ ('Kumamoto Univ).** Treatment of stiff neck (in Japanese). *Rinsho Seikeigeka* 2007: **42:** 419–23.

Ide J¹, Ushijima F, Mizuta H¹ (¹Kumamoto Univ). Stiff neck (in Japanese). Pain Clinic 2007: **28:** 573-8.

Health-Care Center

Mikio Zeniya, Professor and Director Takashi Wada, Associate Professor Hiroki Takahashi, Assistant Professor Yoichi Sakamoto, Professor Takekazu Onda, Associate Professor

General Summary

In the Shimbashi Medical Checkup Office, our research efforts are focused on lifestylerelated diseases.

Research Activities

Preventing lifestyle-related disease through healthy habits

Our mnemonic or motto for promoting healthy habits is "none of one, less of two, more of three." The "one" behavior is smoking. The "two" things are intake of food and alcohol. The "three" things are exercise, rest, and enjoyable activities. We studied the correlation of these six healthy habits with metabolic syndrome. A questionnaire about compliance with our motto was filled out by 18,139 Japanese patients who visited the Health-Care Center at The Jikei University Hospital for medical health checkups. The prevalence of metabolic syndrome was negatively correlated with the number of healthy habits practiced (20.9% for 0, 17.4% for 1, 15.0% for 2, 13.1% for 3, 10.5% for 4, 8.4% for 5, and 7.2% for 6; p < 0.001). Practicing these six healthy habits may be helpful for preventing metabolic syndrome.

Smoking and metabolic syndrome

Metabolic syndrome develops through the accumulation of excess central obesity and insulin resistance. Smoking also causes insulin resistance to develop. The purpose of this study was to clarify whether smoking is correlated with metabolic syndrome and how long the risk of metabolic syndrome remains elevated after smoking cessation. The subjects were 22,892 Japanese who visited the Health-Care Center at The Jikei University Hospital for medical checkups. Compared with never smokers (n=9,434; 41%), the odds ratio (95% confidence interval) for metabolic syndrome for current smokers (n =7,634; 33%) was 1.20 (1.07–1.35) and that for past smokers (n=5,824; 25%) was 1.21 (1.08-1.37). In current smokers, the odds ratio for metabolic syndrome increased with the number of cigarettes smoked, and rate of metabolic syndrome was significantly higher for subjects who smoked 20 or more cigarettes per day. After quitting, a longer cessation period was related to a decreased risk of metabolic syndrome. However, the risk of metabolic syndrome remained elevated for at least 10 years for subjects who smoked 20 or more cigarettes per day and remained elevated for more than 20 years for subjects who smoked 40 or more cigarettes per day. Past smoking as well as current smoking habits may contribute to the development of metabolic syndrome.

Publications

Wada T, Urashima M, Fukumoto T. Risk of metabolic syndrome persists twenty years after the cessation of smoking. *Intern Med* 2007; **46**: 1079–82.

Wada T, Urashima M, Fukumoto T, Joki M, Hashimoto H, Oda S. Effective prevention of metabolic syndrome: "A motto for healthy habits- none of one, less of two more of three". Obest Res Clin Prac 2007; 1: 133-8.

Oikawa T, Takahashi H, Ishikawa T, Hokari A, Otsuki N¹, Azuma M¹ (¹Dept Mol Immunol, Graduate Sch, Tokyo Med, Dent Univ), Zeniya M, Tajiri H. Intrahepatic expression of the co-stimulatory molecules programmed death-1, and its ligands in autoimmune liver disease. Pathol Int 2007; 57: 485-92.

Premedical Course

Japanese

Ikuko Noro, Associate Professor

General Summary

Suitability of informed consent documents for patients written in Japanese

1. The necessity of studying the suitability of informed consent documents for patients written in Japanese was analyzed.

2. A survey was performed to study lay comprehension of readability assessment and feeling of ease with informed consent documents. A total of 363 laypersons, who were not engaged in any medical field, answered a comprehension test and assessed the document after reading it.

Research Activities

Medical interview in Japanese

1. A textbook to analyze medical conversation with the Roter Interactional Analysis System, a method widely used in Western countries, was published.

2. A book written in English on the relationship between medical conversation and patient outcomes was translated into Japanese.

Publications

Noro I, Nakazato Y. The necessity to study suitability of informed consent documents for patients written in Japanese. Ars Linguistica 2007; 14: 73-82.

Reviews

Noro I, Abe K, Ishikawa H. The Roter method of interaction process analysis system (RIAS). Nagoya: Sankeisha; 2007.

Social Science (Law)

Ryuichi Ozawa, Professor

General Summary

Problems of constitutional law in present-day Japan.

Research Activities

I am examining issues related to constitutional law in present-day Japan, especially

pacifism, parliamentary democracy, the welfare state, the right of free speech, and the judicial system. I reported at a seminar entitled "Unequal Society and Constitutional Law" at the Unequal Society and Safety-Net working party of the Legal Committee of the Science Council of Japan.

Publications

Ozawa R. Argument over article 9 reformation of Japanese constitution, past and present. J Jpn Sci 2007; **42:** 36–41. **Ozawa R.** Démocratie représentative et gouvernement d'opinion, VIIème Séminaire Franco-Japonais de droit pubric, Université Montpellier1 C.E.R.C.O.P Université Strasbourg III Institut de Recherche Carée de Malberg, Novembre 2007, pp. 91-95.

Ozawa R. The right of collective defence in post war. In: Rekishi-Kyouikusha-Kyougikai editor. Annuals of historic education and sociologic education. Tokyo: Sanseidou; 2007. p. 56–66. **Ozawa R.** Issues over the Japanese constitution. *Hibakusha problem Rev* 2007; **12:** 1–15.

Human Science

Takao Fukuyama, Professor

General Summary

The study of modern German philosophy and ethics.

Research Activities

I have been working with other Japanese scholars of philosophy to prepare a Japanese translation of Jurgen Habermas's anthology, *between Naturalism and Religion*, which is to be published in 2009. I worked on the chapter "Religious Tolerance as a Pacemaker of Cultural Rights." Jurgen Habermas is a leader of the Frankfurt School, which was founded 1924 and studies the relations of the economy, culture, and the individual in a comprehensive program, called "critical theory." Habermas recently published *Facticity and Validity*, in which he searches for the ethics of discussion in the constitutional state and emphasizes the importance of solidarity in democracy.

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 Margaret Paston and the Celys in the fifteenth century. Ohara also continued a study of how to make useful digital images and XML files of fifteenth century manuscripts, especially of the *Cely Letters*. The results of this investigation were discussed in papers presented at a 2 international conferences.

Fujii took an active research approach to find ways of making college English learners autonomous. He designed a vocabulary-building program that makes students learn and use 2,000 basic-level words and accesses their continual vocabulary development. He also created an extensive reading program that motivates learners to read 1 English book a week from a selected library, exposing learners to a variety of topics and having them write book reports.

Research Activities

Ohara presented a paper at a session entitled "It's the Little Things that Count" at the International Medieval Congress 2007 held at the University of Leeds in the United Kingdom. In this historical sociolinguistic study of the *Cely Letters*, Ohara focused on the usages of auxiliaries and showed how they differ. Ohara also read a paper at the 20th anniversary meeting of the Text Encoding Initiative Consortium at the University of Maryland. In this paper, Ohara focused on whether an electronic text of the letters of Margaret Paston compiled on the basis of the Text Encoding Initiative P5 guidelines can have a useful structure for graphological study and whether it is appropriate to change this kind of text into a more machine-readable one at the cost of complexity and nonlinearity.

Fujii reported on the evaluation and application of his research in a presentation entitled "Designing an Extensive Reading and Vocabulary Building Program" at the 46th national convention of the Japan Association of College English Teachers in Hiroshima, in September 2007.

German

Yoshiaki Shirasaki, Associate Professor

General Summary

German language

I have continued educational activities for medical students and nursing students for the purpose of verbal and nonverbal communication. These activities are also connected with the aim of the development of moral and philosophical ability in the field of intercultural relationships. The numbers of activities per year were 120 for medical and 60 for nursing students.

Swiss literature in the 19th century

I have continued a collaborative study of modern Swiss literature (with the Researching Society for the Helveticum). This study is necessarily related to the preceding study of literary realism but also concerns the modern Swiss-German problem as an aspect of the expression of the German language.

Schiller study

Friedrich Schiller has been regarded merely as a representative poet of German classicism or as a typical dramatist of German idealism. I intend to show his versatility by using a comparative method that would clarify his various influences upon European literature and political history.

Noh and German literature

Noh, a traditional theatre form in Japan with a consistent style and high artistry, includes a general character that is understood in the West. Noh is traditionally discussed in relationship with modern European theatres, and Paul Claudel and Yeats have also produced some great works that show Eastern influences. However, in addition to direct connections, the forms of both noh theatres and Baroque theatre can be identified in German literature, such as the works of Brecht and Hofmannsthal. Here, I intend to clarify the relationship between Noh and Baroque theatre through structural comparison.

Mathematics

Kanji Suzuki, Professor

General Summary

Mathematical Education

At regular meetings of the Mathematics Education Society of Japan, we have proposed several improvements in the methods for teaching mathematics. The contents are as

follows.

1. The arrangement and summation of the methods of teaching calculus, which we have recently published.

2. Some proposals in fields other than calculus included: 1) that in general linear groups, the continuity of inverse operations may be introduced explicitly rather than implicitly by a brief inequality, and 2) in the Chinese remainder theorem appearing in elementary integer theory, the solution of a system of congruence equations may be obtained automatically without difficulty.

Research Activities

1. We provided a new proof of Taylor's theorem in 2001. Using this proof, we can develop a theory of differential calculus, separately from integral calculus.

2. For example, in solving the system of congruent equations $X\equiv 2 \pmod{3}$. $X\equiv 3 \pmod{5}$, $X\equiv 2 \pmod{7}$, we have shown an automatic algorithm leading to the solution $X\equiv 23 \pmod{105}$. Consequently, students may easily learn the whole theory of congruent expressions with the first order.

Publications

Suzuki K. Improvements on teaching methods of calculus, I (in Japanese). Tokyo: Summer Workshop of Mathematics Education Society of Japan; 2007, 7.

Suzuki K. Chinese remainder theorem: teaching by an automatic algorithm (in Japanese).

Tokyo: Mathematics Education Society of Japan (a special edition); 2007, 9.

Suzuki K. A brief inequality: on the continuity of inverse matrices (in Japanese). Tokyo: Mathematics Education Society of Japan (a special edition); 2008, 3.

Physics

Koichi Satoh, Professor

Katsumi Kasono, Assistant Professor

General Summary

Dipalmitoylphosphatidylcholine (DPPC) membranes have been studied from several points of view, including formation, liquid crystals, phase transitions, interaction with ions, and optical characteristics.

Research Activities

Ripple phase of the DPPC membrane

We have analyzed previously published articles about the ripple phase and have found that our analysis of recent research on the dielectric behavior of DPPC vesicles at phase transitions supports our model of the ripple phase.

Monte Carlo simulations of the q = 10,20 Potts models

We have performed cluster update simulations to study systems with the first-order phase transition. We obtained transition temperatures, energies, and order parameters that were consistent with theoretical predictions.

Chemistry

Tomoyoshi Takahashi, Professor

Chikao Hashimoto, Associate Professor

General Summary

The research works of our laboratory are focused on the synthesis of biologically active compounds and the modification of synthetic reactions.

Research Activities

Reaction of α -halo ketone with 2-aminothiol

We previously reported a new type of condensation between α -bromosteroidal ketones and 2-aminothiol which leads to α -ketospirothiazolidine derivatives. In the resulting product, the oxo group migrated to the position originally occupied by the bromine atom. Moreover, to clarify the generality of migration for the oxo group, the reaction of cyclic and acyclic α -haloalkanones with aminothiol yielded the corresponding thiazolidine derivatives. In addition, we found that the use of microwaves improves yields and shortens the reaction time.

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 synthesis of N-protected peptide acids in organic solvents using alkaline earth metal-carboxylate salts of an amino acid was investigated. We found that the amino acid-Ca carboxylate salts are the most effective of the carboxylate salts of amino acids tested for coupling with Boc-amino acid active esters in organic solvents, such as dimethylformamide and dimethylsulfoxide.

Publications

Matsushita M^1 , Takahashi T, Utsukihara T^1 , Shimizu Y^1 , Jansen RJ^1 , Horiuchi CA¹ (¹Rikkyo Univ). Reaction of α -halo-ketone with 2aminothiol: a new synthesis of thiazolidines with the oxo group migrated to the original position occupied halogen atom. *Tetrahedron* 2007; **63:** 8932–8.

Itoh K¹, Utsukihara T², Funayama K², Sakamaki H¹ (¹Nihon Univ), Kanamori M², Takahashi T, Saitoh Y², Matsushita M², He L², Hashimoto C, Sugiyama T (Kyoto Univ), Horiuchi, C A² (²Rikkyo Univ). Reaction of a,b-unsaturated ketones using cerium (IV) sulfate tetrahydrate in acetic acid. Appl Organometal Chem 2007; **21:** 1029– 32.

Hashimoto C, Takeguchi K¹, Kodomari M¹ (¹Shibaura Inst Techo). Synthesis of *N*protected peptide acids using alkaline and alka-
line earth metals-carboxylate salts of amino acids. *Peptide Sci* 2007; 179-82.

Reviews and Books

Hashimoto C. Gel chromatography. Isolation of cholesterol and separation-identification of phospholipid from egg yolk. Isolation of albu-

Biology

Osamu Terasaka, Professor

by electrophoresis. (in Japanese). In: Takahashi T, Shairyoza E (Nihon Univ), Tanaka M (Nihon Med Univ), Yamakura F (Juntendo Univ), editors. Seimeikagaku notameno Kagakujiken. Tokyo: Tokyokyogaku Sha; 2007. p. 160-7, 168-76, 181-92.

min from the white of an egg and its identification

Rie Hiratsuka, Assistant Professor

General Summary

The main research subject of our laboratory is the reproductive system of seed plants. Our research is focused on the relation between pollen tube growth and the programmed cell death of pollen tube conducting tissue.

Research Activities

Distribution of molecules involved in angiospermous pollen tube elongation in the nucellus of gymnosperms

Angiospermous pollen tubes growing into the styles are supplied with carbohydrates and glycoproteins, such as pectins and arabinogaractan proteins (AGPs), from stylar tissue as factors of nutrition, adhesion, or guidance. In this study, we analyzed the localization of starch granules, pectins, and AGPs in the nucellus of *Ginkgo biloba* L., *Cryptomeria japonica* D. Don, *Chamaecyparis pisifera* (Sieb. & Zucc.) Endlicher, *Platycladus orientalis* (L.) Franco, *Pinus densiflora* Sieb. & Zucc., and *Ephedra sinica* Stapf to clarify the role of nucellar tissue in pollen tube elongation.

We found that starch granules were widely distributed throughout the whole nucellus of *P. orientalis and E. sinica* but not at all in *C. pisifera* or *G. biloba*. In *P. densiflora*, starch granules accumulated in the nucellar cells around the tube tip but were markedly decreased in the nucellar cells through which the pollen tube passed. In *C. japonica*, starch granules were located only in the mid-zone of the nucellus.

Esterified pectins were located in the cell walls and extracellular matrix within the nucellus of all materials. The pectins drastically increased during programmed cell death of nucellar cells in response to pollen tube elongation in *P. densiflora*.

AGPs were present in the cell wall and extracellular matrix of the whole nucellus in *C. japonica, C. pisifera, P. orientalis, and P. densiflora.* In *P. orientalis* and *P. densiflora,* AGPs were abundant in the nucellar cells around the pollen tube.

These findings suggest that the molecules that are involved in angiospermous pollen tube elongation are also present in the nucellus of gymnosperms and that their distribution pattern varies among species.

School of Nursing

Basic Nursing I

Sawako Haga, Professor Mayumi Kikuchi, Assistant Professor Machiko Hirao, Associate Professor

Research Activities

The research activities of the basic nursing group can be divided into the following 3 areas.

Haga has been investigating the effects of physical assessment by nurses, the evidence of nursing skill, and the history of nursing.

Hirao has been investigating the history of nursing and Nightingale's thoughts about nursing.

Kikuchi has been investigating the effects of nursing education, teaching methods, and nursing diagnosis.

Reviews and Books

Onoda T supervised, Takahashi A, Haga S, Sato F, editors. Practice! Physical assessment (in Japanese). 3rd ed., Tokyo: Kanehara; 2007.

Haga S, Hirao M, Ebina F. A study of Dr. Kanehiro Takaki's thought on health education (2): from his opinions related to the improvement of high school and university education (in Japanese). J Jpn Soc Med Hist 2007; 53: 70-1.

Haga S, Hanyu C Kikuchi M, Hirao M, Aoki N. The practice of the physical assessment's lecture (in Japanese). Kango Jinzai Kyouiku 2007; 4: 107–19.

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Basic Nursing 2

Sugino Oishi, Professor

General Summary

To clarify the characteristics of the Japanese nursing system after World War II, I have been studying the history of nursing and analyzing the factors affecting the Japanese nursing system.

Research Activities

Analysis of factors affecting the Japanese nursing system

The purpose of this study was to analyze changes in the numbers of nurses and assistant nurses, the ratio of the number of nurses to that of the combined total number of nurses and assistant nurses (nurse ratio), and the factors that caused the changes from 1998 through 2004 in Japan. For these analyses, official dates were used.

The results were as follows. 1) From 1998 through 2004, nurse ratios increased in all prefectures. 2) The nurse ratio and number of nurses in each facility depended on the structure of the population and the medical system. 3) Differences in nursing structures between prefectures were affected by the percentage of elderly persons and economic conditions.

I presented the results in my lecture on informatics science.

Study of the policy of the Nursing Affairs Division GHQ by analyzing censorship by GHQ

Records of the censorship of medical and nursing publications from 1945 through 1949 in occupied Japan are thought to be housed in the Gordon W. Prange Collection at the University of Maryland.

The purpose of this study was to investigate the circumstances of publishing and the criteria for censorship by General Headquarters Supreme Commander for the Allied Powers (GHQ) in occupied Japan. We visited the Gordon W. Prange Collection and collected and analyzed nursing books, nursing magazines, nursing newspapers, and censored documents of the Gordon W. Prange Collection.

The results were as follows. 1) The Nursing Affairs Division of GHQ censored the Japanese Nurses Association's journal before it was censored by the Civil Censorship Detachment of GHQ. 2) GHQ's position on censorship evolved during the occupation. 3) Statistics on publication, circulation, and subscribers of Japanese nursing magazines were discovered.

Historical research on Japanese nursing reform after World War II

The Nursing Affairs Division of GHQ revised the nursing law and the nursing system during the occupation. The present Japanese nursing system is based on the nursing

reforms of GHQ but has been modified because of economic and educational problems. I obtained information from the GHQ records and related persons to investigate nursing reform.

I introduced the methods of historical investigation and presented the results of my study to students in my lecture on nursing management.

Publications

Oishi S. Quantitative analysis of the changes in the number of nurses and assistant nurses in Japan from 1998 to 2004 (in Japanese). *Tokyo Jikeikai Med J* 2008; **123**: 15–25.

Oishi S, Kita K, Hirao M, Haga S. Censorship in occupied Japan(3): "Sukoyaka". the Journal of the Public Health Nurse's Association in Nagano.

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Reviews

Oishi S. Home care nursing (in Japanese). *Jpn J Home Care Nursing* 2007; **12:** 388–94.

Adult Nursing

Shoko Fujino, Professor

Chie Watanabe, Assistant Professor

General Summary

We have studied the acquisition of nursing skills in clinical adult practice. We examined the achievement of nursing skills in nursing having some experience in adult nursing practice.

We investigated what nursing skills graduates had wanted to study while they were students at our university. Many nurses wanted to learn how to dispense medicines to patients and how to manage drip infusions. We increased the number of classes teaching these skills.

Research Activities

Fujino examined effective touch techniques by nurses for palliative care. The results were classified and described in 17 concepts and 11 categories. When the nurses touched to the patient in palliative care, it was very important with the observation of "Cueing" sign from patients. The touches that the patient expected and accepted, kept the comfort of patients. We called the touching as "Caring touch".

Watanabe performed a nationwide survey about information given by oncologists regarding chemotherapy-related infertility. On the basis of these results, we published a booklet about sexuality and fertility issues after chemotherapy for cancer survivors. We have developed guidelines on nursing care for ambulatory chemotherapy. We are also planning a study evaluating preventive intervention for postoperative lymphedema.

Publications

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Reviews

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Fujino S. CPR (in Japanese). In: Basic technique of nursing. 2nded. Tokyo: Igakugeijutsusya; 2007. p. 578–605.

Fujino S. Bed sore (in Japanese). In: Basic technique of nursing. 2nded. Tokyo: Igakugeijut-susha; 2007. p. 634-43.

Fujino S. Cancer nursing (in Japanese). In: Basic technique of nursing. 2nded. Tokyo: Iga-kugeijutsusha; 2007. p. 644-59.

Fujino S. Teaching in nursing practice (in Japanese). In: Basic technique of nursing. 2nd ed. Tokyo: Igakugeijutsusha; 2007. p. 660-73.

Fujino S. Terminal care (in Japanese). In: Basic technique of nursing. 2nded. Tokyo: Iga-kugeijutsusha; 2007. p. 684-90.

Watanabe C. Stoma care (in Japanese). In: Basic technique of nursing. 2nded. Tokyo: Iga-kugeijutsusha; 2007. p. 182-7.

Yokoi S. Cardiac catheterization test (in Japanese). In: Basic technique of nursing. 2nd ed. Tokyo: Igakugeijutsusha; 2007. p. 382-91.

Miyoko Sakurai, Professor

Kumiko Date, Associate Professor

General Summary

The main research interest of our division is nursing intervention for elderly persons with chronic health problem and for their family members.

Research Activities

Study of education about pressure ulcers

The main subject in our division is studying ways to prevent pressure ulcers in elderly persons. We began an investigation of basic nursing skills and education about pressure ulcers.

Research on the fatigue of family caregivers for elderly persons with dementia Sakurai et al. have been investigating the psychological conflicts of family caregivers for elderly persons with dementia.

Relationship between health and lifestyle in elderly adults

Date has been investigating the health status and various factors influencing it among middle-aged and elderly adults for primary and secondary prevention of lifestyle-related diseases from a comprehensive perspective, including nutrition, exercise, and rest.

Mental Health and Psychiatric Nursing

Setsuko Hayashi, Assistant Professor

Research Activities

I studied the educational relationships between teachers and nursing students with mental health problem and then investigated nurses' awareness of psychiatric nursing practice.

This time, I investigated nurses' hesitation about and awareness of nursing practice in psychiatric wards, which have recently included an increasing number of private rooms. As a result, by being one patient per one life space, the nurses became aware of the ease of stabilizing the mental status of patients, the ease of caring for the needs of each patient, and the accessibility of psychiatric practice for citizens. In contrast, the nurses became aware of the difficulty of watching the practice of other nurses, of keeping a suitable distance from patients, and of protecting patient's privacy, and then hesitated.

Publications

Hayashi S, Teraoka T (Keio Univ, Graduate Student), Ikebe T (Yokohama City Univ). Nurses' hesitation and awareness to nursing practice in the psychiatric wards that consists of private room (in Japanese). *Nippon Seishin Hoken Kango Gakkaishi* 2007; **16:** 67–74.

Child Nursing

Kiyo Hamanaka, Professor

Kayo Cho, Assistant Professor

Research Activities

The present conditions and problems of basic education in child nursing Hamanaka presented results of a study from the previous year at the conference of the Japanese Society of Child Health Nursing. The results will be applied to a future curriculum.

The present conditions and problems of postgraduate education in child nursing Hamanaka submitted for publication the results of a study conducted the year before last. Development and verification of educational support programs for continuing work in the clinical area of child care

A study of the organization of practice in nursing with outpatients and outpatient nursing to promote the health of children in basic education

Hamanaka started a study of the organization of practice in nursing with outpatients and outpatient nursing to promote the health of children in basic education and carried out a training program based on a tentative plan.

As a co-author of a study with the support of a Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Hamanaka presented results of a study from last year at 2 congresses and chaired a session at the annual meeting of the Society of Ambulatory and General Pediatrics of Japan. In addition, Hamanaka twice performed focus group interviews about nursing practice for outpatients. In addition, a program for health promotion was reviewed.

The social growth process of children with end-stage renal disease and drafting a support plan by offering information

Cho interviewed children with end-stage renal disease and their parents to clarify methods to deal with problems that patients and parents face in the process of social growth. This research is supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology.

Publications

Hamanaka K., Hanazawa Y. The current status of and issues concerning new staff and preceptors related to postgraduate education and guidance in the field of child nursing: from an investigation undertaken at a general hospital. *J JSCHN* 2008; **17:** 31-7.

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

Menstrual symptoms and their relationship with women's perception on health, gender and own physique A study of 394 female students collected data on menstrual symptoms and their relationship with women's perception of health, gender, and their own physiques. The data indicated that women recognized themselves as the greatest factor affecting health. Although 93.5% of the subjects had a body-mass index of 25 kg/m² or less, 40.1% of subjects considered themselves to be "slightly overweight" or "overweight." The relationship between individual factors and menstrual symptoms is being analyzed and is to be presented to the relevant academic society following more detailed analysis.

Health issues and nursing assistance during adolescence

Unwanted pregnancy, sexually transmitted diseases (STDs), self-harm behavior, and malignant tumors have been examined as typical health issues of adolescence. In the international comparison of awareness of the prevention of unwanted pregnancy and STDs, Japan showed a low level of consciousness of preventing STDs and a high level of perception that men should initiate contraception despite the high rate of sexual experience among the Japanese population. The study of trends in self-harm behavioral research identified an increase in the number of cases reported in the psychiatric literature and an increase in the number of cases that involved difficulty in handling patients since 1996. An interview with an adolescent girl with ovarian cancer found that the patient was undergoing treatment with support from her parents while suffering from inner conflict between the emotional reliance on her parents and the adolescent desire for independence.

Bacteriological study of nosocomial infections from the molecular epidemiological and drug sensitivity approaches

MecA-positive *Staphylococcus aureus* and *S. aureus* resistant to vancomycin, and linezolid, and methicillin, which are especially problematic causes of nosocomial infections, were analyzed in detail using molecular epidemiological and drug-sensitivity approaches. Many of these bacteria are already resistant to antibacterial drugs currently used against methicillin-resistant *S. aureus*. It is necessary to monitor their future trends.

Publications

Hososaka Y, Hanaki H¹, Endo H¹, Suzuki Y¹, Nagasawa N (Kyushu Univ), Otsuka Y (Soc Insur Central Gen Hosp), Nakae T¹, Sunakawa K¹ (¹Kitasato Inst). Characterization of the oxacillin-susceptible *mecA*-positive *Staphylococcus aureus*: a new type of MRSA. J Infect Chemother 2007; **13**: 79-86.

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Reviews and Books

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Community Health Nursing

Noriko Okuyama, Professor

Miki Shimada, Associate Professor

General Summary

The major research projects in our department have been focused on: 1) the development of teaching materials, 2) learning achievement degree of the public health nurse and 3) establishing a community-based end-of-life care system through the organization of social capital.

Research Activities

Community health nursing education

In a society with an aging population and a falling birthrate, the primary requirements for nurses engaged in community health nursing are quick response, collaboration, ethics, morals, and, most importantly, the ability to respond to drastically changing social demands. The aim of this research was to examine whether early exposure is an effective learning method for student nurses in community health nursing education.

Establishing a community-based end-of-life care system through the organization of social capital

The purpose of this study was to identify the components necessary to establish a care system, such as a mutual regional support mechanism, understanding by the general public, and network structures, focusing on social capital, to play a role in the establishment of a community-based end-of-life care system.

Publications

Yoshioka Y (Tsukuba Univ), Kinoshita Y (Ibaraki Pref Coll), Okuyama N. A study about the study start time of community health nursing in a first degree. J Jpn Acad Commun Health Nurs 2008; **10:** 94–100.

Home Care Nursing

Masako Sato, Associate Professor

Hiromi Kasuga, Assistant Professor

General Summary

Home care nursing is the field that concerns patients who receive medical treatment at home, families who care for patients, and the nursing support needed by patients and their families.

Research Activities

Research on an e-learning system for nursing students

To plan a seminar and practicum effectively in a limited time, we used the "Moodle" e-learning system, which is a management system for online courses, as a self-learning tool. We obtained some data about using this system for nursing students. In future studies, we will explore the possibilities of using this system in various situations for the study of home care nursing.

Research about cancer patients who die at home and their families

We investigated the amount of money spent for treatment by the families of patients who died of cancer at home. We also investigated the feelings of the families and what support visiting nurses provided to the families of patients with terminal cancer who received medical treatment at home.

Study of the structure of mental experiences of patients with progressive, intractable disease based on the analysis of their diaries

We clarified the mental experience of patients with amyotrophic lateral sclerosis, as an example of a progressive, intractable disease. We performed a qualitative analysis of the diaries written by a patient with amyotrophic lateral sclerosis.

Study of changes in the thoughts of citizens concerning medical services in a municipality under rehabilitation

In a municipality under rehabilitation, we studied changes in the citizens' thoughts about medical services, after privatization of the local hospital. We will continue this research in the future, too.

Publications

Sato M, Kasuga H. The study about structure of mental experience with progressed intractable disease based on the analysis of their diaries (in Japanese). *Jpn J Health Sci (Gunma Counseling Assoc)* 2007; **11**: 51–6.

Reviews

Kasuga H. The pioneer of home care nursing the 12th series (in Japanese). *J Home Care Nursing* 2007; 4.

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