Research Activities

2018

The Jikei University School of Medicine

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Introduction

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

The Jikei University established the Centre for International Affairs in April 2015 to further promote its international activities. The University has sent 24 students abroad and received more than one hundred overseas elective students in the academic year 2018. We have partnerships with 12 universities worldwide. A number of researchers are also doing joint research in laboratories all over the world. I hope that *Research Activities* will promote the University's contribution to the global society.

We owe much to the efforts of Professors Michihiro Yoshimura and Masao Okazaki, and the members of the Academic Information Center in editing this report.

Senya Matsufuji
President
The Jikei University School of Medicine

November 1, 2019

Academic Information Center

Susumu Minamisawa, *Director*
Masao Okazaki, *Professor*
Ruri Ashida, *Professor*

General Summary

The Academic Information Center, which consists of the Library Services Section (including the Shimbashi Main Library and the Kokuryo Branch Library), the Medical Museum, the Photography Room, the Historical Collection Room (*The Jikei Historical Collection*), the Medical English Laboratory, and the Centre for International Affairs, aims to support the activities for education, research, and medical care of this university by providing comprehensive access to academic resources.

Research Activities

The Academic Information Center supports research activities performed in this university by the following characteristic functions of each section.

Library Services Section
Library services are operated by 2 libraries: the Shimbashi Main Library (Nishi-Shimbashi Campus) and the Kokuryo Branch Library (Kokuryo Campus).
Since our Library Services began in 1885 at "Sei-I-Kwai Bunko" (our libraries' origin), we have been maintaining a collection of books and journals. As of March 31, 2019, the number of books and journals held is 262,872 by the Shimbashi Main Library and 92,648 by the Kokuryo Branch Library.
With the prevalence of academic e-resources, the libraries have replaced print journals with e-journals. In 2018, more than 8,400 foreign e-journals were accessible through the title-list menu on the libraries' website. As for e-resources, the libraries also subscribed to bibliographical and clinical decision support databases and maintain a linking system that leads users from the search results of databases to full texts or relevant information. To support the use of e-resources for research activities, the libraries always update users' manuals on the website and offer hands-on seminars on a regular and on-demand basis (63 participants in 2018).
Because e-journals and databases are accessible within the campus network, a remote access system is implemented for this university's researchers to log in from the outside (315 newly registered remote-access users in 2018).
The Library Services also include editorial roles for journals published by the university: *Jikeikai Medical Journal*, *Tokyo Jikei-kai Ika Daigaku Zasshi* (in Japanese), *Research Activities*, and *Kyoiku Kenkyu Nenpou* (in Japanese).
Along with managing their journals, editorial committees of *Jikeikai Medical Journal* and *Tokyo Jikei-kai Ika Daigaku Zasshi* jointly held lectures in May, in which the topics were related to writing English papers by the following lecturers: Prof. Fusao Kato (Division of

Neuroscience, Research Center for Medical Sciences) (May 10), Prof. Hiroshi Yoshida (Department of Laboratory Medicine) (May 17), Prof. Masao Okazaki (Academic Information Center) (May 24), and Prof. Osamu Ohara and Assistant Prof. John Surya (May 31).

To raise the awareness of publication ethics among researchers and postgraduates, The University introduced a plagiarism detection tool, *Turnitin*, in 2016, and the libraries are assigned to receive and answer inquiries concerning *Turitin*. Libraries also prepared the *Turitin*'s user registration form on their website and registered 63 new personal users in 2018.

The libraries maintain an institutional repository, *Academic Repository of The Jikei University School of Medicine*, to preserve the academic outputs of this university and to make them available on the Internet. In 2018, 472 articles, including the university's publications and doctoral theses, were registered in the Academic Repository.

To understand the recent movement of encouraging accessibility to research results, the libraries held the "Open Science Seminar" on January 15 (35 participants). In the seminar, Senior Project Manager Koichi Ojiro of the Research Center for Open Science and Data Platform of the National Institute of Informatics suggested the universities' roles for managing experimental data in their institutional repositories in correspondence with the prospective open science framework that is now in preparation at the National Institute of Informatics. Concerning the open access, a member of the Academic Center also made a presentation on how to avoid submitting manuscripts to so-called predatory journals, or pseudo-journals.

Medical Museum

The Medical Museum is basically designed for students' self-study, but it is also used for research purposes.

In the Medical Museum, 1,824 gross specimens and 2,549 microscopic specimens (as of March 31, 2019) are displayed by the topics that include Dr. Higuchi's Collection of Ovarian Tumors, Dr. Kameda's Gallstone Collection, and the Molded Dried Specimen Collection. In 2018, the Medical Museum renovated 42 specimens by removing stains and renewing the explanations.

From January 21 to February 1, the Medical Museum held a poster exhibition entitled "*Sogo Tenji*" at the Nishi-Shimbashi Campus, featuring research activities of 4 of the retiring professors: Prof. Sadamu Honma (Division of Oncology, Research Center for Medical Sciences), Prof. Iwao Ohno (Division of General Medicine, Department of Internal Medicine), Prof. Seiji Isonishi (Department of Obstetrics and Gynecology), and Prof. Hiroshi Sekine (Deprtment of Radiology).

In addition, to support in-house research meetings, the Medical Museum keeps and lends desks, chairs, signboards, and amplifier equipment.

Photography Room

The Photography Room supports researchers preparing presentation materials by taking photographs of specimens and patients' lesions or by shooting videos of research topics. In addition, the Photography Room modifies photographic and video image files for man-

uscripts or presentation forms. Large-format and other types of color printers are available in the Photography Room.
In 2018, the Photography Room dealt with 5,380 photographs and 11,394 color prints (including posters).

Historical Collection Room (The Jikei Historical Collection)
The Historical Collection Room collects and keeps historical materials related to this university and to founder Dr. Kanehiro Takaki. In the exhibit space, paintings and photographs are arranged in chronological order together with other historical materials with brief descriptions.
The Historical Collection Room also cooperates in historical research by showing materials it holds or by referring to other related documents and resources. In 2018, the Historical Collection Room received 90 visitors and 86 inquiries.

Medical English Laboratory
Masao Okazaki, *Professor*
The Medical English Laboratory is a section responsible for education and research related to medical English. The section supports research activities of this university by editing English journals published by this university; helping researchers improve posters, presentations, and articles submitted to journals; and giving lectures on writing papers and making presentations in English.

Centre for International Affairs

Ruri Ashida, *Professor and Director*

General Summary

The Jikei Centre for International Affairs (JCIA) was established in the Academic Information Center in April 2015 to promote international exchange and enhance globalization of The Jikei University School of Medicine. Some of the activities of the JCIA include: (1) English education of medical students, nursing students, doctors, nurses, and other members of the hospital staff; (2) selecting and sending students to partnership (and non-partnership) universities abroad for their clinical electives; (3) accepting and organizing rotations for elective students from abroad; (4) holding seminars and workshops to enhance global perspectives; and (5) promoting collaborations with partnership universities abroad. JCIA established its own office with a lounge for Jikei and international students to communicate.

Research Activities

1. Professor Ashida held simulation sessions with English-speaking simulated patients

for medical and nursing students, doctors, and nurses at The Jikei University and other universities (University of Tokyo, Tokyo Medical University, Toho University, Showa University, St. Luke's International University, Akita University, and Tohoku Medical and Pharmaceutical University) and studied the effects of working with English-speaking simulated patients.

2. Professor Ashida invited Dr. James S. Newman and Torrey A. Laack of Mayo Clinic, and held a "boot camp." It consisted of a faculty development session on teaching through simulation and a simulation session for students.

3. Supported by scientific research funding from the Ministry of Education, Culture, Sports, Science and Technology, Professor Ashida held educational simulations in the ER using multi-cultural scenarios to develop doctors' communication skills in caring for foreign patients. Dr. James S. Newman was invited again to hold a two-day simulation session.

Publications

Ashida R, Fukuda K, Minamisawa S, Oishi K. Medical electives abroad: Do they contribute to the "globalization" of physicians? *J Med Eng Educ.* 2018; **17:** 40.

Continuing Medical Education Center
The Continuing Medical Education Committee

Masahiro Abo, *Director*
Yasuo Toriumi
Tatsuhiro Joki
Keizo Takagi
Rimei Nishimura
Masanori Takeishi

General Summary

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

Research Activities

1. Registered members: 173 (as of April 1, 2019)
Members using the Center: 290/year
2. The 39th Tokyo Jikei Medical University − University Hospital Medical Cooperation Forum was held on August 4, 2018. A total of 204 persons participated.
3. Monthly seminars were held on the afternoon of the second Saturday of the month in April, June, November, and February. Each seminar was attended by 15 to 25 persons.
4. The “CME Center News” is mailed monthly to the registered members.

Center for Medical Education

Osamu Fukushima, *Professor and Director*
Mariko Nakamura, *Professor*
Fumiko Okazaki, *Assistant Professor*
Hisashi Onoue, *Professor*
Yoshio Ishibashi, *Associate Professor*

General Summary

The Office of Educational Development was founded in 1999. Staff members were recruited from the School of Medicine. Its main interests were (1) the analysis of medical education reports published by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), the Ministry of Health, Labour and Welfare (MHLW), and medical associations; (2) the technical support of faculty and the management of faculty development and education seminars; and (3) the implementation of tutorials, objective structured clinical examinations, and community-based medical education programs in the undergraduate curriculum. However, many improvements have been required in our undergraduate medical and nursing education, postgraduate clinical training programs, and continuing professional development for healthcare workers. In 2005, the office of Educational Development was reorganized as the Center for Medical Education. Furthermore, the secretariat was set up in the Center in 2006. The bylaws for the Center were revised in 2011, 2013, and 2015.

The Center now consists of the Branch for Physician Professional Development Support, the Branch for Nursing Professional Development Support, the Branch for Simulation Education, the Branch for Community-based Medical Education and Research, the Branch for Educational Institutional Research, and the Branch for Administration. The Branch for Physician Professional Development Support is subdivided into the Office of Undergraduate Medical Education and the Office for Educational Development. The Branches contribute to undergraduate educational activities in medical and nursing schools and practical nursing schools; staff development in the university and 4 attached hospitals; and the management of an e-learning system and simulation training centers for students, faculty, and staff in attached hospitals and healthcare providers in the community.

Research Activities

1. Regarding undergraduate and graduate educational activities, Professor Fukushima was the course director of Medicine in General III to VI; the educational unit director of the Community Service for the Handicapped Program (year 1), the Care for Severely Handicapped Children and Incurable Patients Program (year 2), the Support for Child Rearing in the Community Program (year 2), and the Practical Training on Elderly Medical Care Experience Program (year 3); and the elective educational unit director of Primary Care in the Community and Regional Hospitals (years 1 to 6) and Medical Research (years 1 to 6). Professor Nakamura was the educational unit director of Introduction to Health Care

Practice (year 1 of medical and nursing students) and Medicine in General Practice III (year 3). Professor Onoue was the educational unit director of Basic Clinical Skill Training Program (year 4) and Clinical Tutorial Series (years 4 and 5). Assistant Professor Okazaki was the educational unit director of Health Care at Home (year 3), Working at a Hospital Program (year 3), Family Medicine Practice (years 4 and 5), and Medicine in General Practice IV (year 5). Regarding graduate school education, Professor Fukushima, Professor Nakamura, and Professor Onoue were in charge of medical education in the common graduate school curriculum.

2. The Branch for Nursing Professional Development Support organized seminars for education nurses, nursing educators, nurse managers, and nurses involved in the end of life.

3. The Branch for Educational Institutional Research analyzed entrance examination data, students' performance data, and graduate questionnaire data for implementation of educational activities.

4. The Branch for Simulation Education carried out maintenance of equipment at the simulation center for improvement of the educational environment for undergraduate and postgraduate students and members of the hospital staff.

5. The Office for Educational Development engaged in planning and operation of a forum for medical education leaders (hosted by the Japan Medical Education Foundation) and a seminar for medical and dental education leaders (hosted by MEXT).

6. Professor Fukushima participated in third-party evaluation activities of vocational education in practical schools that were supported by MEXT.

7. As a participant in the activities of the Japan Accreditation Council for Medical Education, Professor Fukushima served as a chief evaluator in external evaluation teams at Fukuoka University and Ehime University and as an evaluator at Tokushima University. Professor Nakamura served as a chief evaluator at Miyazaki University and as a deputy at Kanazawa Medical School. Professor Nakamura was a lecturer in the evaluator training workshop held by the Japan Accreditation Council for Medical Education.

8. As a participant in the activities of the Common Achievement Tests Organization, Professor Nakamura was a member of several computer-based testing (CBT) committees and was sent as a CBT monitor to Yamagata University, Kobe University, and Fukui University. Associate Professor Ishibashi was a member of several CBT committees and was sent as a CBT monitor to Teikyo University. Assistant Professor Okazaki was a member of several objective structured clinical examination (OSCE) committees and was sent as an OSCE monitor to Osaka City University, Kansai Medical College, Jichi Medical School, and Niigata University.

9. Contribution to other institutions of higher education (faculty development lectures and workshops): National Defense Medical College; Gifu University; Japan Association of Judo-Seifuku Colleges; teacher training for judo therapists held by the MHLW; teacher training for occupational therapy, physical therapy, and speech therapy held by the MHLW, IMS Group Patient Safety, Mejiro University, Hyogo Medical School Hospital, and Fukushima Medical College.

10. Regarding faculty development activities on campus, Associate Professor Ishibashi participated as a facilitator or member of the chief task force in faculty development (FD)

for clinical clerkship teachers, FD for clinical supervisors in postgraduate clinical training, and FD for OSCE evaluator training at years 4 and 6. Assistant Professor Okazaki participated as a facilitator or member of the chief task force in FD for health providers in the community, FD on active learning introduction, FD for clinical clerkship teachers, FD for clinical supervisors in postgraduate clinical training, and FD for OSCE evaluator training at years 4 and 6.

11. As a participant in the activities of the Japan Council for Evaluation of Postgraduate Clinical Training, Associate Professor Ishibashi served as an evaluator at NTT Medical Center Tokyo and Tokyo Rosai Hospital.

12. Regarding postgraduate clinical education activity, Associate Professor Ishibashi was in charge of the Endoscopic Surgical Training Course at The Jikei University Hospital.

Department of Anatomy (Gross Anatomy and Neuroanatomy)

Yoshinori Kawai, *Professor* Toru Hashimoto, *Assistant Professor*

General Summary

Our department's research activities have focused on neuroanatomy and gross anatomy. In neuroanatomical research, organizations of neuronal networks and their development are investigated with morphological and electrophysiological methods to elucidate brain function and diseases. Our primary interests are the quantitative architecture and dynamics of neural circuits and their relationships. In gross anatomical research, the functional importance of variations of organ systems is explored in human cadavers and animals.

Research Activities

To integrate and broadcast neural information, local microcircuits and global macrocircuits interact within certain specific nuclei of the central nervous system. The structural and functional architecture of this interaction was addressed for the caudal nucleus of the tractus solitarius (NTS), a relay station of peripheral viscerosensory information processed and conveyed to brain regions concerned with autonomic–affective and other interoceptive reflexive functions.

Differential ascending projections from the caudal NTS: an interface between local microcircuits and global macrocircuits

To integrate and broadcast neural information, local microcircuits and global macrocircuits interact within certain specific nuclei of the central nervous system. The structural and functional architecture of this interaction was addressed for the caudal NTS, a relay station of peripheral viscerosensory information. Axon collaterals of most small NTS cells establish excitatory or inhibitory local microcircuits likely to control the activity of nearby NTS cells and transfer peripheral signals to efferent projection neurons. At least 2 types of cells that constitute efferent pathways from the caudal NTS were distinguished: (1) greater numbers of small cells, seemingly forming local excitatory microcircuits via recurrent axon collaterals, that project specifically and unidirectionally to the lateral parabrachial nucleus and (2) much smaller numbers of cells that likely establish multiple global connections, mostly via the medial forebrain bundle or the dorsal longitudinal fascicle, in a wide range of brain regions, including the ventrolateral medulla, hypothalamus, central nucleus of the amygdala, bed nucleus of the stria terminalis, spinal cord dorsal horn, brainstem reticular formation, locus coeruleus, periaqueductal gray, and periventricular diencephalon (including the epithalamus).

The evidence presented here suggests that distinct caudal NTS cell types distinguished by projection patterns and related structural and functional features participate differentially in the computation of viscerosensory information and the coordination of global macro-

networks in a highly organized manner.

Publications

Kawai Y. Spatiotemporal Structure and Dynamics of Spontaneous Oscillatory Synchrony in the Vagal Complex. *Front Neurosci.* 2018 Dec 18; **12:** 978. doi: 10.3389/fnins.2018.00978. eCollection 2018.
Kawai Y. Differential Ascending Projections From the Male Rat Caudal Nucleus of the Tractus Solitarius: An Interface Between Local Microcircuits and Global Macrocircuits. *Front Neuroanat.* 2018 Jul 24; **12:** 63. doi: 10.3389/fnana.2018.00063. eCollection 2018.

Department of Anatomy (Histology and Embryology)

Masataka Okabe, *Professor*
Hideaki Suzuki, *Assistant Professor*
Hisashi Hashimoto, *Professor*
Yasuyo Shigetani, *Assistant Professor*

General Summary

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

Research Activities

Effect of buffer composition in fixative on immunogenicity
Antibodies do not always show immunopositive reactions on a tissue section prepared from a tissue fixed with 4% paraformaldehyde (PFA) in sodium phosphate buffer (SPB). In this situation, some pretreatment to improve the penetration of the antibodies or retrieve to the antigenicity might be attempted. Moreover, a fixative free of PFA might be challenged. In this study, we have attempted the immunostaining on a tissue fixed with 4% PFA in HEPES buffer and obtained immunopositive reactions with antibodies that had shown no immunoreaction when fixed with 4% PFA in SPB. These antibodies were directed against Ca-binding proteins in the nervous system and membrane proteins. Phosphate ion in 4% PFA in SPB might precipitate in a tissue by binding with calcium ion, and the precipitate might become an obstacle for antibody penetration. Alternatively, structural changes in 3-dimensional conformation of a protein, which might be induced by deprivation of calcium ion by phosphate ion in 4% PFA in SPB, resulted in a loss of antigenicity. Although not all antibodies show immunopositive reactions on tissue fixed with 4% PFA in HEPES, this fixative deserves consideration for some antibodies.

Regeneration of basal lamina associated with neuromast formation during posterior lateral line development in Polypterus
The genus *Polypterus*, the most basal extant actinopterygian fish in molecular phylogeny, is reminiscent of coelacanth because of scales covered by dentin and enamel. We focused on the development of the neuromast closely related to the lateral line scale during posterior lateral line development in *Polypterus*.
Initial neuromast cells appeared as the cranial placodes in the neurula and migrated to the caudal side within the lower epidermal layer adjacent to the horizontal septum in the larva. The cell population migrated, depositing a set of neuromast cells to form a rosette-like structure, and finally reached the caudal fin while repeating this process. The basal lamina with periodic acid-methenamine silver staining, also obvious with scanning elec-

tron microscopy, did not exist just underneath the neuromast, and neurites from the neuron bundle stained with neuron-specific antibodies innervated to the neuromast cells within the epidermis. The neuron bundle away from the neuromast was clearly seen underneath the basal lamina and was continuous to the cranial ganglion, suggested as the lateral line nerve. The lateral line nerve thus innervated to the neuromast cells within the epidermis outside the basal lamina, which was regenerated, where is not directly below the neuromast during posterior lateral line development.

Functional analysis of mouse glial cells missing family genes

Mammals have 2 members of the glial cells missing (Gcm) family of transcription factors — Gcm1 and Gcm2 — which are expressed in different regions and play important roles in developmental regions. The gene glial cell missing transcription factor 1 (*Gcm1*) is expressed in mice during placental development, and a deficiency of this gene in mice causes placental hypoplasia and embryonic lethality. The *Gcm2* gene is expressed in the parathyroid developing area, and deficient mice die soon after birth owing to calcium dysregulation. Because deletions of the Gcm family are lethal, to analyze the function of the postnatal Gcm family, a conditional deletion method is essential. We constructed mice in which the DNA binding region was sandwiched between LoxP sequences for functional analysis in Gcm1 and Gcm2 and performed functional analysis of these mice by crossing them with region-specific and time-specific Cre mice.

1. Mouse *Gcm1* has been shown to be expressed in the kidney and the placenta, and in-situ hybridization has confirmed its expression in the renal tubular area of the kidney. Therefore, to clarify the function of Gcm1 in the kidney, we used a mouse (*Wt1-Cre* × *Gcm1*$^{Flox/Flox}$) specifically deficient in *Gcm1* in the metanephric mesenchyme from which the renal tubule was derived. We found that the *Gcm1*-deficient kidney showed no change in size, shape, or function, but when ischemic injury was given, *Gcm1*-deficient kidney showed reduced cell proliferation and fibrosis marker genes. These results suggest that the function of Gcm1 in the kidney is involved in the recovery from ischemic injury.

2. Mouse *Gcm2* is expressed in the parathyroid gland throughout life; this gene is involved in the development of the parathyroid gland developmental stage, but its postnatal function is unknown. Therefore, to clarify the Gcm2 parathyroid function in adult mice, we performed tamoxifen-induced time-specific *Gcm2* deletion analysis. When tamoxifen was administered to 8-week-old mice (*Tm-Cre* × *Gcm2*$^{Flox/Flox}$) to make *Gcn2* deficiacy in parathyroid. At 1 month of *Gcm2* deficiency in the parathyroid gland, changes were not seen in the size, expression of related genes, calcium concentration in the blood, but some follicular structures were observed. Seven months after the deletion of *Gcm2*, the parathyroid gland had shrunk, resulting in decreased expression of related genes and a decreased calcium concentration. The follicular structures seen at 1 month of *Gcm2* deficiency parathyroid were more marked, and the shape had also significantly changed. When cell proliferation and cell death were observed, the cell proliferation decreased from 1 month after the defect, and at 7 months cell proliferation decreased and cell death increased. These results clearly show that Gcm2, which is expressed in the parathyroid gland throughout life, is involved in maintaining cell proliferation, shape, and function of the parathyroid gland even after birth.

Analysis of the origin of the gas bladder

To investigate how fish acquired a gas bladder, we analyzed sturgeon, gar, zebrafish, and *Polypterus*. The comparative analysis showed that the branching region of the gas bladder from the esophagus is different in each fish. The gas bladder of gar was branched from the entrance of the esophagus (larynx in mammals), the same as the lung of *Polypterus*. The gas bladder of zebrafish branched from the central part of the esophagus, and the sturgeon gas bladder was branched from the gastric entrance. In analysis of enhancers related to lung development, while this region is conserved in *Polypterus*, insertion and deletion of sequence have already been observed in gar, is hardly conserved in zebrafish, and is completely defective in sturgeon. These results show that the gas bladder can be acquired in various ways. The gar gas bladder seems to use the lung mechanism. Zebrafish and sturgeons have lost lung developmental mechanisms, which might have caused the development of gas bladders in the posterior region of the esophagus. This is an important finding for understanding the relationship of lungs to the gas bladder in regard to gas bladder acquisition.

Functional analysis of microRNA-155 in dextran sulfate sodium-induced mouse model of colitis

Ulcerative colitis is a refractory disease for which maintaining long-term remission improves quality of life. However, long-term remission has been maintained in few cases with the current treatment method, and a new treatment method is greatly desired. At this time, C57BL (wild-type; WT) mice, which are more sensitive to dextran sulfate sodium (DSS), and microRNA (miR)-155 knockout mice, which are less sensitive, were prepared as mouse models of DSS-induced colitis, which are commonly used as mouse models of ulcerative colitis, to clarify the key molecular mechanisms of preventing the occurrence and recurrence of colitis. When the mice were analyzed, exfoliation of epithelial cells, loss of crypts, and significant inflammatory cell infiltrate in the lamina propria were observed in WT mice as a pathological change in large-intestinal mucosa due to DSS administration. However, no tissue damage was observed in miR-155 knockout mice.

Next, the real-time polymerase chain reaction method was used to measure the expression level of miR-155 before and after colitis was induced in WT mice. An increase in the miR-155 expression level was observed after DSS was administered. As a result, a correlation between miR-155 and the occurrence and worsening of colitis was indicated. Currently, miR-155 expression was verified by using in situ hybridization before and after DSS administration in WT mice has been verified using the in-situ hybridization method. Based on data obtained with this experiment, we would like to clarify the homeostatic mechanism of large-intestinal mucosa and to verify whether inhibition of miR-155 can be achieved with a new treatment method to maintain remission of ulcerative colitis.

Joint morphogenesis in zebrafish fins

In tetrapod limbs, synovial joints are important structures for locomotion. Fish fins are thought to be homologous to tetrapod limbs, but in the fin ray region are unique joints, which are filled with ligament-like connective tissues. To elucidate the mechanism of the fish joint formation, we have observed a series of transverse sections with hematoxylin,

Alcian blue, and periodic acid–Schiff stain. We found that an articular cavity, which was the region positive for hematoxylin and Alcian blue, was in the joint architecture. Moreover, the proteoglycan 4a gene (*prg4a*) was expressed at the fin joint. These findings suggest that the fin joint is classified into a diarthrosis, although it consists of the articular capsule and a mucin-containing cavity but not cartilage.

Publications

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Hirasaki Y, Tomita T, Yanagisawa M, Ueda K, Sato K, Okabe M. Heart Anatomy of Rhincodon typus: Three-Dimensional X-Ray Computed Tomography of Plastinated Specimens. *Anat Rec (Hoboken).* 2018; **301:** 1801-8.

Suzuki H, Nishida H, Kondo H, Yoda R, Iwata T, Nakayama K, Enomoto T, Wu J, Moriya-Ito K, Miyazaki M, Wakabayashi Y, Kishida T, Okabe M, Suzuki Y, Ito T, Hirota J, Nikaido M. A Single Pheromone Receptor Gene Conserved across 400 My of Vertebrate Evolution. *Mol Biol Evol.* 2018; **35:** 2928-39.

Yamada T, Tatsumi N, Anraku A, Suzuki H, Kamejima S, Uchiyama T, Ohkido I, Yokoo T, Okabe M. Gcm2 regulates the maintenance of parathyroid cells in adult mice. *PLoS One.* 2019; **14:** e0210662.

Nagasawa T, Kawaguchi M, Yano T, Isoyama S, Yasumasu S, Okabe M. Translocation of promoter-conserved hatching enzyme genes with intron-loss provides a new insight in the role of retrocopy during teleostean evolution. *Sci Rep.* 2019; **9:** 2448.

Department of Molecular Physiology

Shigeru Takemori, *Professor*
Toshiko Yamazawa, *Associate Professor*
Maki Yamaguchi, *Associate Professor*
Tetsuo Ohno, *Assistant Professor*

General Summary

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

Research Activities

Effect of exercise on ingested polyamine accumulation in various tissues of rats

Polyamines, such as putrescine, are polycation molecules indispensable for cell proliferation. Polyamines are also reported to modulate cardiac excitability through the gating of ion channels and to modulate cell viability through autophagy induction. We have recently found that exercise suppresses putrescine accumulation in the heart and serum of rats ingesting putrescine. Here, we examined the effects of exercise on ingested putrescine accumulation in other tissues. Six-week-old female Wistar rats were fed with 1 mg/ml drinking water for 9 weeks with or without free access to a wheel for spontaneous running. The skeletal muscle, liver, lung, spleen, and fat of these rats were then dissected for polyamine content analysis with high-performance liquid chromatography. Urine adsorbed on filter papers covering the cage bottom was also analyzed for polyamine metabolites. Exercise significantly suppressed putrescine accumulation after ingestion in other tissues tested. Although tissue levels of spermidine did not differ significantly in rats whether ingesting putrescine or exercising, we suspect that the ingested putrescine might be rapidly turned over because the spermidine, which is synthesized from putrescine in cells, increased in the urine. Because exercise might affect the metabolism of putrescine and its turnover, more comprehensive analysis of metabolites and stable-isotope tracer analysis by mass spectrometry are needed.

Functional analysis of skeletal muscle type ryanodine receptor carrying malignant hyperthermia associated mutations

In excitable cells, membrane depolarization is translated into intracellular Ca^{2+} signals, and ryanodine receptors (RYRs), located in the sarcoplasmic/endoplasmic reticulum membrane, play a key role in intracellular Ca^{2+} release. There are 3 isoforms of RYRs, of which RYR type 1 (RYR1) is dominantly expressed in skeletal muscle. Mutations of the ryanodine receptor 1 gene (*RYR1*) cause severe muscle diseases, such as malignant hyperthermia, which is a disorder of Ca^{2+}-induced Ca^{2+} release via RYR1 in skeletal muscle. Thus far, more than 300 mutations of *RYR1* have been reported in patients with malignant hyperthermia, and most mutations have been found in 3 “hotspot” regions. However, because the structure-function relationship of mutant RYR1 has not been comprehensively analysed, the mechanism remains largely unknown.

Here, we combined functional studies and molecular dynamics simulation of RYR1 bearing disease-associated mutations at the N-terminal region. When expressed in HEK293 cells, the mutant RYR1 caused abnormalities in Ca^{2+} homeostasis. Molecular dynamics simulation of the mutant RYR1 revealed that alterations of hydrogen bonds/salt bridges between N-terminal subdomains strongly correlate with the channel function of RYR1. In particular, mutations of R402, which play a key role in connecting the 3 subdomains (A, B, and C) of the N-terminal region, cause clockwise rotations of B and C subdomains with respect to the A subdomain. This movement might increase the probability the channel is open and provides the structural basis of malignant hyperthermia in R402 mutants.

Differential scanning calorimetry measurement of water components in skinned skeletal muscles

Magnetic resonance images reflect water content and water states in tissue. By taking advantage of well-organized skeletal muscle, we have recently clarified that magnetic resonance can be used to distinguish localized water clusters of 5 states. Although each water state is considered to reflect protein-water interactions mutually restricting their motional freedom, the nature of each water state has not been clarified in detail.

To obtain information about water states from different aspects, extra heat absorption of skeletal muscle fibers was measured with differential scanning calorimetry to detect the "melting" of ice; the motional freedom of fibers would be restored at a characteristic temperature absorbing extra heat.

With differential scanning calorimetry, skinned fibers of sartorius muscle from *Rana catesbeiana* at rigor condition showed extra heat absorption at −24˚C, −21˚C, 0˚C, 46˚C, and 65˚C. The peak at 46˚C and 65˚C would represent denaturation of myosin and actin filament, respectively, because selective removal of myosin or actin filaments diminished the corresponding peaks, and the temperature values are close to those reported for the denaturation of corresponding proteins from rabbit psoas.

The denaturation and selective removal of myosin and actin filaments differentially affected the peaks at −24˚C and −21˚C. The peak at −24˚C was affected mainly by the manipulation of actin filaments, and the peak at −21˚C was affected by both myosin filaments and actin filaments.

Finally, we confirmed the difference of the thermal stability of skinned fibers between rigor and relaxed states, because experiments conducted so far have been performed exclusively in the rigor state. As a result of heating for 60 minutes at 30˚C in each condition, the contractile force of the skinned fiber in the both the rigor and relaxed states was reduced to 80% before heating. These findings suggest that trends similar to those of the rigor state can be obtained in the relax state.

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Department of Cell Physiology

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Yoichiro Kusakari, *Associate Professor*
Masato Konishi, *Visiting Professor*
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General Summary

The aim of research in our laboratory is to understand the regulatory mechanism of the cardiovascular system. In particular, we are interested in the development of the cardiovascular system, the mechanics of sarcomere contraction, Ca^{2+} homeostasis in the cardiac sarcoplasmic reticulum, and the pathophysiology of cardiac fibrosis and pulmonary hypertension. We established an experimental system to investigate small fetal arteries, such as the rat fetal ductus arteriosus (DA) and pulmonary veins. In addition, we developed an *in vivo* nanoimaging system to observe sarcomere contraction in the ventricles of small animals, such as rats and mice.

Research Activities

Analysis of characteristics of pulmonary veins
Pulmonary veins must differ in character from systemic veins because they carry blood with a high concentration of oxygen. However, the characteristics of pulmonary veins remain a mystery. We successfully established a novel, feasible rat model of pulmonary hypertension due to left heart disease by generating left atrium stenosis and are analyzing the effects of pulmonary congestion on the vascular structure and the function of pulmonary veins. In addition, we have generated the atrium-specific overexpression and deletion of pituitary homeobox 2c, a transcription factor that is specifically expressed in the pulmonary veins and the left atrium.

Molecular mechanism of closure of the DA
The DA is an essential artery that connects the main pulmonary artery and the descending aorta in the fetus. The DA closes immediately after birth in accordance with its smooth muscle contraction and vascular remodeling. We found that prostaglandin E_1 induces the secretion of the protein nephroblastoma overexpressed (NOV or CCN3) in rat DA smooth muscle cells and that this protein inhibits intimal cushion formation of the rat DA. We have also done the comprehensive analysis of the gene expression of the chicken DA and the aorta. These projects are in collaboration with Waseda University.

Regulation of endoplasmic reticulum stress on cardiac function
Endoplasmic reticulum (ER) stress is associated with cardiac function. We are interested in the regulation of the serine/threonine protein kinase/endoribonuclease inositol-requiring transmembrane kinase/endonuclease 1α (IRE1α) on ER stress in heart failure. in the sarcoplasmic reticulum. We found that IRE1α induced transient ER stress signaling and

conferred a protective effect against pressure overload-induced pathological remodeling in the heart. This project is a collaborative work with Professor Yibin Wang at the University of California, Los Angeles.

Regulation of cardiac metabolism
Cardiac metabolism plays an essential role in maintaining cardiac function. In the damaged heart, fatty acid (FA) oxidation is impaired and glycolysis is promoted. We examined the regulation of cardiac metabolism in a mouse model of cardiac injury due to the administration of monocrotaline. Metabolites in the tricarboxylic acid cycle were decreased and those in glycolysis were increased at 6 weeks. We found that pyruvate dehydrogenase activation is an early event to compensate for a subtle metabolic impairment from myocardial damage.

Pathophysiological mechanisms of overstretch-induced cardiac dysfunction
The mechanism of impaired cardiac function in a volume-overloaded heart is incompletely understood. We studied the effect of diastolic overstretch on cardiac function in isometrically contracting muscle prepared from rat right ventricular papillary muscles. We found that acute, severe overstretch of an isolated rat papillary muscle causes the inner collapse of mitochondria with the sarcomere structure being preserved. Therefore, we believe that abrupt disruption of the mitochondrial structure by acute diastolic overstretch might account for the mechanisms on pathogenesis of acute volume-overloaded heart failure.

Mechanism of sarcomere contraction in cardiac muscle
Sarcomeric contraction in cardiomyocytes serves as the basis for the heart's pump functions. Although sarcomeres play a pivotal role in the circulatory system, changes of myocardial sarcomere length have not been systematically investigated *in vivo*. Here we developed a high-speed (100 frames per second), high-resolution (20 nm) spinning disc confocal-imaging system for the beating mouse heart *in vivo*. Via expression of α-actinin-*Aequorea coerulescens* green fluorescent protein under this optics system, we analyzed physiological sarcomere dynamics in a single myofibril consisting of approximately 30 sarcomeres (i.e., with a near entire length) in a ventricular myocyte, simultaneously with hemodynamic variables (i.e., electrocardiography, left ventricular pressure, and a pressure volume loop). The findings were as follows. First, the sarcomere length values were 1.88 ± 0.29 μm in diastole and 1.66 ± 0.19 μm in systole, and the individual sarcomere length values varied markedly during the cardiac cycle, even in the same myofibril. Second, the dynamic behavior of each sarcomere was not always synchronized with that of an entire myofibril. Third, the correlation (R) between the dynamics of an individual sarcomere and that of an entire myofibril varied markedly, i.e., from −0.2 to 0.8, during 6 cardiac cycles. Fourth, sarcomeres that actively contributed to myofibrillar dynamics and those that did not coexisted at a similar ratio. These findings provides new insights for our understanding of cardiac function at the single-sarcomere level.

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Department of Biochemistry

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General Summary

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

Research Activities

Forced expression of dual-specificity tyrosine-regulated kinase 2 exerts antitumor effects via apoptotic induction in liver cancer

Liver cancer is highly aggressive and globally exhibits a poor prognosis. Recently, tyrosine kinase inhibitors, including sorafenib, lenvatinib, and regorafenib, have been developed and approved for clinical use against advanced cancers but have limited effects. Therefore, novel molecules that can become targets for future therapies should be quickly identified.

We have reported that dual-specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2) functions as a tumor suppressor by regulating cell survival, differentiation, proliferation, and apoptosis. However, the precise functional roles of DYRK2 in liver cancer remain obscure. Moreover, research into the clinical application of DYRK2 as a molecular target remains to be performed.

We assessed the effects of DYRK2 on tumor growth and apoptosis in liver cancer cells. We showed that DYRK2 knockdown enhances cell proliferation and tumor growth through the expression of c-Myc and cyclin D in liver cancer cells. Conversely and more importantly, adenovirus-mediated overexpression of DYRK2 inhibits cell proliferation, tumor growth, and the induction of apoptosis both in vitro and in vivo. Furthermore, we found that patients with liver cancer and low DYRK2 expression survived for a significantly shorter time. The findings that DYRK2 regulates proliferation and apoptosis of cancer cells suggest that DYRK2 expression is a promising marker for prognosis in liver cancer. Stabilized or forced expression of DYRK2 might thus become a target for novel gene therapy against liver cancer.

Study of DYRK2 expression and stability

Our previous studies have suggested that DYRK2 has a tumor-suppressive function. For

example, immunostaining analysis has shown that DYRK2 is down-regulated in several tumor tissues compared to adjacent normal controls. Therefore, if DYRK2 expression can be restored, it might be used for cancer therapy, but the mechanism of DYRK2 expression in cells is poorly understood.

The promoter region of the *DYRK2* gene containing of high guanine-cytosine (GC) content suggests an expression control mechanism of messenger (m) RNA by DNA methylation. On the other hand, within DYRK2 mRNA is a 5′-untranslated region (5′-UTR) with high GC content over 300 bases upstream of the start codon, and the upstream open reading frame (uORF) is present there. These features suggest the existence of a translational regulatory mechanism via the 5′-UTR of mRNA. Therefore, the influence of this 5′-UTR on the expression of firefly luciferase was analyzed with a simian virus 40 (SV40) promoter-driven reporter assay system. The 5′-UTR starting from the transcription start site of the *DYRK2* gene was amplified by the polymerase chain reaction and inserted between the SV40 promoter and the luciferase reporter gene. As a result, the activity of luciferase was reduced 80%. When the uORF region was deleted from the sequence inserted into the reporter vector, the luciferase activity was restored to half of its original level. These results suggest that translation regulation via 5′-UTR exists as a regulation mechanism of DYRK2 expression.

The expression level of DYRK2 in cells is also regulated by a protein degradation system. The relation of its domain structure to intracellular stability has been investigated in *Drosophila* DYRK2 but not in human DYRK2. On the other hand, the crystal structure of the kinase domain of human DYRK2 has been elucidated, which is consistent with the region reported previously. Here, we forced expression of the human DYRK2 kinase domain in human cell lines and assessed its expression level by immunoblotting. We found that the level of expression of the kinase domain of human DYRK2 in cells was much lower than that of the full-length DYRK2. When several deletion mutants were made and their expression levels were compared, the N-terminus was found to greatly contribute to intracellular stability and to be conserved from human to zebrafish. In contrast, the C-terminal region following the kinase domain contributed little to the stability of DYRK2.

Molecular functions of DYRK2 during mammalian tissue development

Tissue development proceeds via spatiotemporal patterning of several signaling molecules. These signaling molecules are regulated by posttranslational modifications, such as phosphorylation, in addition to gene expression. We have reported that among these post-translational modification-related factors that the dual-specificity tyrosine-regulated kinase 2 gene (*Dyrk2*) is a key regulator of p53, which it phosphorylates at Ser46 to induce apoptosis in response to DNA damage. However, little is known about the molecular functions of DYRK2 when mammalian tissue develops. In this study, we aimed to identify the molecular functions of DYRK2 in tissue development. For this purpose, we have established methods related to immunohistochemistry, immunocytochemistry, *in situ* hybridization, and *in vitro* cultivation. With these methods, we would like to identify target-signaling molecules of DYRK2.

Subcellular localization of serine/threonine kinases

Intracellular kinases are key factors involved in the intracellular signaling pathway. Our laboratory has paid attention to some serine/threonine kinases. In this study, we analyzed the association between kinases and cancers.

After performing intracellular localization analysis of kinases, we found that a novel kinase X was located both inside and outside of cells. For our experiment systems, we have used biochemical, cellular biological, and immunologic techniques. Furthermore, our research, which was done in collaboration with the Department of Internal Medicine of The Jikei University Hospital, detected kinase X at a high level in the serum of patients with cancer. In addition, we found that kinase X bound to the surface of cancer cells. Thus, from this study, we have clarified the novel localization of kinase X and its association with cancer. We are planning future studies to elucidate the basic biological and clinical significance of this kinase.

Provirus integration site for Moloney murine leukemia virus 1 regulates self-renewal property of colorectal cancer cells by regulating Akt/mechanistic target of rapamycin pathways

Provirus integration site for Moloney murine leukemia virus 1 (Pim-1) is a proto-oncogenic kinase involved in several cellular processes, including cell survival, cell proliferation, and apoptosis. Increased Pim-1 expression is frequently observed in cancer cells and is correlated with a poor prognosis in various types of cancer. Accumulating evidence has demonstrated that cancer stem cells (CSCs) are a small subpopulation of cancer cells that possess stem-like properties. To enrich CSCs, a functional approach is the sphere culture system, which provides self-renewal ability. Although CSCs are associated with the maintenance and growth of tumors, the cellular signaling pathways that regulates the capacity of CSCs has not been fully understood. In this study, we have shown that Pim-1 function is required for self-renewal capacity in colorectal cancer cells. Our results demonstrated that Pim-1 expression is elevated in sphere-forming cells. Depletion of Pim-1 or treatment with the Pim inhibitor SGI-1776 prevented sphere formation. Furthermore, inhibition of Pim-1 prevented phosphorylation of Akt and ribosomal protein S6 in sphere-forming cells. These findings suggest that Pim-1 contributes to the self-renewal property of colorectal CSCs by maintaining Akt and mechanistic target of rapamycin (mTOR) signaling.

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Department of Molecular Biology

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General Summary

Our research has focused on the biological significance of regulating cellular polyamines, in particular through the polyamine-regulating protein antizyme (AZ). Polyamines are ubiquitous biogenic amines that are essential for cell proliferation and are related to various phenomena, such as differentiation, development, cancer, and autophagy. The latest works have shown that polyamines have significant effects on longevity, memory, and arteriosclerosis. Three major polyamines — putrescine, spermidine, and spermine — are present in mammalian cells. When cellular polyamines increase, AZ is induced through a translational frameshift. After being induced, AZ binds to ornithine decarboxylase, a key enzyme for polyamine biosynthesis, and inhibits the enzymatic activity and accelerates degradation of the enzyme protein. Thus, AZ provides the feedback regulation for cellular polyamine levels. Mammalian cells express 3 members of the AZ family (AZ1-3), and each AZ is likely to have a specific function.

Functional significance of interaction between MYCN and AZ2 in neuroblastoma cells
We have previously found that AZ2 interacts with MYCN, which is highly expressed in neuroblastoma and is a poor prognosis factor for patients. AZ2 accelerates MYCN degradation in ubiquitin-independent manner. So far, knocking down AZ2 in neuroblastoma cells with small interfering RNA has increased colony formation in soft agar more than two-fold compared with that in control cells. This year we performed xenograft mouse model analysis with AZ2 knocked down BE (2)-C cells to confirm the tumor formation at the whole-body level. As expected from colony formation assay, 24 days after nude mice had been implanted with AZ2 knocking down cells, tumor volumes had increased 7-fold and weight had increased 4-fold compared with those in control cells. These results strongly indicate that AZ2 is involved in tumor growth in neuroblastoma. We are studying the detailed mechanism of AZ2-mediated suppression of tumor growth and crystal structure analysis of the AZ2-MYCN complex for drug development.

Analysis of interaction between AZ and ATP citrate lyase
We identified ATP citrate lyase (ACLY), a cytosolic enzyme that catalyzes the production of acetyl-CoA and is used for lipid anabolism and the acetylation of cellular components, by screening for AZ-binding proteins. We have recently reported that AZ1 and AZ2 bind to and activate ACLY in cancer cells. Although AZ is a negative regulator of cellular polyamines, how the activation of ACLY by AZ is related to polyamine metabolism is unclear. A likely hypothesis is that acetyl-CoA produced by ACLY from citrate in the cytoplasm facilitates the acetylation of polyamines, and, as a result, the export of intracellular acetyl-polyamines is increased. To confirm this hypothesis, intracellular and extra-

cellular acetyl-polyamines under the culture of ACLY overexpressed EXOD-1 cells (a variant cell line isolated from murine breast cancer cells) were measured and compared with control cells. In ACLY overexpressed EXOD-1 cells, both intracellular and extracellular acetyl-polyamines were significantly increased. This result suggests a relationship of ACLY activity to intracellular polyamine acetylation. We are studying the effects of ACLY or AZ knockdown on the levels of intracellular and extracellular acetyl-polyamine in EXOD-1 cells to clarify the mechanism in detail.

Analyses of physiological roles of AZIN1

Antizyme inhibitor (AZIN) 1 is a catalytically inactive homolog of ornithine decarboxylase which positively regulates cellular polyamines by inhibiting AZ. We have previously shown that AZIN1-deficient mouse embryonic fibroblasts (M-MEFs) have the features of growth inhibition and morphological changes, such as the appearance of cells having multinuclei, a macronucleus, or a micronucleus. To address the cause of these phenomena, we focused on intact M-MEFs (iM-MEFs) and intact W-MEFs (iW-MEFs) having no nucleus morphological changes. We measured the centrosome number in iM-MEFs and iW-MEFs by means of antibodies for gamma-tubulin as the centrosome marker. Centrosome numbers were lower in iM-MEFs than in iW-MEFs. Considering our previous report that AZIN1 colocalizes with Az1 mainly at centrosomes during the period from prophase through late anaphase, these results suggest that the defects of AZIN1 lead to a fault of chromosomal segregation mediated by gamma-tubulin and to morphological changes, such as multinuclei or a macronucleus. Our findings suggest that AZIN1 is an important factor for proper cell-cycle progression.

Translation efficiency affects the sequence-independent +1 frameshifting by polyamine

Using a human cell-free translation system, we have shown that ribosomal +1 frameshifting occurred in both a sequence-independent and a polyamine-dependent manner. To gain insight into the mechanism of this +1 frameshifting, we investigated the relationship of efficiencies of +1 frameshifting to translation. To evaluate the translation efficiency of messenger RNA sequences, we used a codon adaptation index (CAI) for human genes. The CAI is an effective measure of synonymous codon usage bias. The index is evaluated within the range of 0 to 1. A CAI range of 0.8 to 1 represents high translation efficiency. The CAI range of transcription factor + 36 nucleotides (TF+36nt) (TCCTTCTGCTCTTTCAGCCAACTTATTCTACTCCGACGATCGGCT, from the readthrough region of the human AZ1 gene) is 0.50, and this value represents a low translation efficiency. Substitution of 7 codons in the TF+36nt sequence with synonymous codons of higher CAI increases the CAI value to 0.86 (TCCTTCTGCTCTTTCAGCCAgCTcATTCTgCTgCGgCGgTCcGCT, substituted nucleotides were indicated by lowercase letters). These substitutions reduced the efficiency of +1 frameshifting compared with the original TF+36nt sequences. Conversely, substitution of 6 codons in CAT45 (AACGTGGCCAATATGGACAACTTCTTCGCCCCCGTTTTCACGATG, from a bacterial chloramphenicol acetyltransferase gene), which has a high CAI (0.86) into synonymous codons of a lower CAI (0.62) (AAtGTtGCgAATATGGACAAtTTCTTCGCgCCgGTTTTCACGATG, substituted nucleotides were indicated by lowercase letters),

increased the probability of +1 frameshifting as compared with the original CAT45 sequences. These results suggest an inversed correlation between the efficiencies of sequence-independent polyamine-induced +1 frameshifting and translation.

Publications

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Department of Pharmacology

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General Summary

The research interests of the Department of Pharmacology include the following.
1. Synaptic transmission and its modulation in the basal ganglia and basal forebrain (Toshihiko Momiyama)
2. Neural control of breathing in aquatic vertebrates (Naofumi Kimura)
3. Peripheral benzodiazepine receptors on adrenal cells (Yuji Ohno)
4. Purinergic receptor-linked up-regulation of melatonin synthesis-related enzymes in mast cells (Haruhisa Nishi)
5. Analysis with optogenetics of cerebrocerebellar interaction (Taro Ishikawa and Misa Shimuta)
6. Mild hypothermia-mediated neuroprotection for experimental ischemia through adenosine receptors (Masahito Kawamura)
7. Nanoscale distribution of synaptic vesicles at a central presynaptic terminal (Yukihiro Nakamura)
8. Cholinergic modulation of central synaptic transmission (Etsuko Suzuki)

Research Activities

Synaptic transmission and its modulation in the basal ganglia and basal forebrain
Electrophysiological studies with slice patch-clamp recording techniques were performed to analyze synaptic transmission and its modulation, mediated by dopamine, serotonin, and muscarine receptors, in the nigrostriatal or mesolimbic dopaminergic system and in the cholinergic system of the basal forebrain. Developmental changes in the modulation are also under investigation. These systems are involved in various psychological functions and in their disorders, including Parkinson's disease and Alzheimer's disease. Furthermore, optogenetic activation techniques for neurons in these brain areas have been introduced to analyze neuron type-specific synaptic transmission and its modulation. These basic analyses can lead to the identification of the mechanisms underlying the related disorders mentioned above and to the development of novel therapeutic tools.

Neural control of breathing in aquatic vertebrates
The neural respiratory output of the isolated brainstem of *Xenopus laevis* displayed 2 motor patterns: the lung ventilation-like large bursts and the functionally unidentified small bursts. The lung ventilation-like bursts were abolished by bath application of the low concentration (0.1 μM) of the μ-opioid receptor agonist DAMGO ([D-Ala(2), N-Me-Phe(4), Gly(5)-ol]-enkephalin) and restored by 1 to 5 μM naloxone. In contrast,

the small bursts were resistant to the low concentration of DAMGO. The small bursts might have a common origin with the buccal rhythm of terrestrial frogs.

Peripheral benzodiazepine receptors on adrenal cells

Peripheral benzodiazepine receptors localize in the outer mitochondrial membrane, transfer cholesterol in steroidogenic organs under physiological conditions, and are readily upregulated under various pathological conditions, such as cancer, inflammation, and neurological disease. We would like to investigate whether endozepine and its metabolite, which we prepared from bovine adrenocortical cells, are related to these pathological conditions.

Purinergic receptor-linked up-regulation of melatonin synthesis-related enzymes in mast cells

Recent investigations of cells involved in human immune system regulation have suggested that mast cells play important roles in maintaining homeostasis. That mast cells are unique in their ability to release melatonin and that melatonin has beneficial antioxidative effects are not widely recognized. Furthermore, the detailed mechanism of melatonin production in mast cells is unclear.

The present study focused on mast cell melatonin synthesis because of its roll in immune responses. Cells of LAD2, a human-derived mast cell line, were analyzed for their expression of both messenger (m) RNA and proteins for the 2 key enzymes of melatonin synthesis: aralkylamine N-acetyltransferase and hydroxyindole O-methyltransfase. The intracellular cascades involved in enzyme synthesis were also examined.

The LAD2 cells were positive for the mRNA expression of both enzymes. The mRNA levels were enhanced by stimulation of the G-protein coupled $P2Y_{11}$ receptor with no degranulation; in contrast, stimulation of the $P2X_7$ receptor (ligand-gated ion channel) did not enhance mRNA levels but did induce degranulation.

These results suggest that melatonin release from mast cells is involved in maintaining homeostasis and is not involved in allergic responses.

Analysis of the cerebrocerebellar interaction using optogenetics

Cerebrocerebellar communication is important in a wide range of brain functions, including sensory information processing. We investigated the somatosensory-signaling pathways to the cerebellar cortex, using transgenic mice whose cerebral cortex can be suppressed by light illumination, and revealed that direct signals from the trigeminal nucleus and indirect signals via the somatosensory cortex are integrated in Purkinje cells and in granule cells in the cerebellar cortex. Recently, we have also found that the primary somatosensory cortex is linked to both the climbing fibers and the mossy fibers projecting to the cerebellar cortex.

Mild hypothermia-mediated neuroprotection for experimental ischemia through adenosine receptors

The therapeutic hypothermia for acute stroke might play an important role in neuroprotection; however, the key mechanism of this therapy is still undetermined. We examined

the role of adenosine in hypothermia-induced neuroprotection by using extracellular and patch clamp recordings. Mild hypothermia (32°C) causes protection for ischemia-induced loss of synaptic transmission through activation of adenosine A_1 receptors, but deep hypothermia (28°C)-induced neuroprotection is not caused by adenosine receptors. This study might reveal the involvement of adenosine in the therapeutic hypothermia (usually 32°C-33°C) for acute stroke.

Nanoscale distribution of synaptic vesicles at a central presynaptic terminal

The probability and timing of neurotransmitter release are determined by the coupling distance between voltage-gated calcium channels and synaptic vesicles. To investigate the spatial distribution of ready-releasable vesicles, we performed simultaneous patch-clamp recording from the calyx of Held and postsynaptic medial nuclei of trapezoid body neurons in acute brainstem slices of mice. Excitatory postsynaptic currents were evoked by strong depolarization and were then analyzed with deconvolution. The comparison of this result with simulations of vesicular release reveals that synaptic vesicles are widely distributed over 10 to 25 nm but are most concentrated around 50 nm from calcium channels in the active zone.

Cholinergic modulation of central synaptic transmission

Acetylcholine is a neurotransmitter involved in learning and memory. In the central nervous system, acetylcholine has been shown by several studies to modulate synaptic transmission and the firing properties of neurons. We elucidated the cholinergic modulation in striatum using an electrophysiological technique. In the striatum, we have found that GABA release from striatal medium spiny neurons onto cholinergic interneurons is inhibited by the activation of presynaptic intracellular muscarinic M1 receptors.

Publications

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Department of Pathology

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General Summary

The objective of our research in the Department of Pathology is to morphologically investigate the causes of disease and to evaluate morphological changes. We use human tissue samples resected at autopsy and surgery or obtained at biopsy. These samples are examined with such means as light microscopy, electron microscopy, morphological measurement, immunohistochemical staining, and molecular pathological techniques.

Research Activities

Research on the Digestive Tract

1. To clarify predictive factors for metastasis to the lymph nodes and the liver in patients with submucosal cancer invading the stomach, we examined 578 cases of intramucosal cancer or submucosal cancer of the stomach which were endoscopically or surgically resected in our hospital from 2004 through 2015. Of these cases, 230 (39.8%) were submucosal cancers. In patients with submucosal cancer, representative sections were stained with elastica-Van Gieson stain, which is a special stain, and immunohistochemical biomarkers (CD31, D2-40, and MIB-1 antibodies). We regarded the following items as metastatic risk factors to perform a multivariate analysis of extracted data: the presence or absence of ulcer, the presence or absence of elevation or excavation, tumor diameter (< 20 mm, ≥ 20 mm), measured infiltration values (< 500 μm, ≥ 500 μm), histologic types at the invasive site, infiltration patterns, lymphovascular invasion (lymphatic invasion [+/−] and venous invasion [+/−]), the locations and numbers of lymphovascular invasion in the primary lesion, and the form of carcinoma in the lymphovascular vessels (individual tumor cells and tumor cell nest formation).

2. To investigate predictors of lymph-node metastasis in patients with submucosal colorectal cancer, we studied specimens obtained from 124 patients with submucosal colorectal cancer from 2009 through 2015 and stained with hematoxylin and eosin. Representative sections underwent special staining and immunohistochemical staining. Data on the following variables were extracted: the presence or absence of depressions, intramucosal growth patterns (nonpolypoid growth, polypoid growth), the measured depth of invasion (< 1,000 μm, ≥ 1,000 μm), histologic type of the invasion site, the presence or absence of tumor budding (+/−), lymphovascular invasion: lymphatic invasion and venous invasion (+/−), the locations and numbers of lymphovascular invasion sites in the primary lesion, the presence or absence of a mixture of poorly differentiated adeno-

carcinoma and mucinous carcinoma, tumor budding, and types of intravascular carcinomas (sporadic tumors, nested tumors). We are planning to perform multivariate analysis in the future.
3. We studied the histologic characteristics of colorectal neuroendocrine tumors. In particular, we examined the relations of tumor grade based on the 2010 World Health Organization Classification of Gastrointestinal Tumors to vascular invasion and outcomes. Surgically and endoscopically resected colorectal carcinoid tumors were stained with elastica–Van Gieson stain, immunostain (CD31, D2–40, and MIB–1 antibodies), and hematoxylin and eosin. Each lesion was classified according to tumor grade, and the tumor diameter, invasion depth, submucosal invasion distance, number of nuclear division cycles, Ki67 index, and lymphovascular invasion were assessed. The relations of lymphovascular invasion and other risk factors to outcomes were then studied. In our hospital, we are currently analyzing data on 160 cases consisting of 139 endoscopically resected cases and 21 surgically resected cases.
4. Two surgically resected specimens of Crohn's disease of the small intestine were all prepared as tissue specimens., mapped the site of epithelioid granuloma and ulcer, and searched for the distribution of granulomas within the wall and the positional relationship between granulomas and ulcers. Of 385 granulomas, 1.3% were in the mucosa, and 19.8% were in the submucosa. Overall, 97.1% of the granulomas were within 10 mm of ulcers in a horizontal direction. To accurately diagnose Crohn's disease, biopsy specimens, including the submucosa, should be taken from regions within 10 mm of ulcers.

Research on the urogenital system
1. In patients who had prostate cancer with a Gleason score of 3 + 4 = 7, we examined whether the proportion of Gleason pattern 4 in biopsy specimens and other biopsy variables are useful for predicting outcomes after total prostatectomy. Patients in whom the proportion of Gleason pattern 4 was 5% or higher in biopsy specimens had higher risks of malignancy and biochemical recurrence than did patients with a Gleason score of 3 + 3 = 6.
2. In 148 patients with urothelial cancer, we examined the relations of the presence or absence of overexpression of human epidermal growth factor receptor 2 (HER2) to the immunohistochemical subtype and clinicopathological factors. Protein overexpression or gene amplification of HER2 was found in 14% of patients. All urothelial cancers were immunohistochemically classified into basal or luminal subtypes. Protein overexpression or gene amplification of HER2 was found in 4% of patients with basal cancers and in 22% of patients with luminal cancers.

Research on the female genital organs
1. To construct a new classification of cervical adenocarcinoma tissue, we participated in an international joint study and obtained the following results.
a. We developed a comprehensive immunohistochemistry algorithm required for the diagnosis of histologic subtypes. The paper was submitted and published in the following journal: Am J Surg Pathol 2018, 42: 989–1000.
b. We demonstrated that invasion patterns can be regarded as predictive factors for

lymph-node metastasis in patients with human papillomavirus (HPV)-related cervical adenocarcinoma but not in patients with HPV-unrelated cervical adenocarcinoma. We submitted a paper to the following journal: Gynecologic Oncology 2018, 150: 56-60.
c. We clarified clinical findings of HPV-related and HPV-unrelated cervical adenocarcinomas and submitted a paper to the following journal: Am J Surg Pathol 2019, 43: 466-474.
d. We examined the histologic, immunohistochemical, and clinical characteristics of cervical squamous-cell adenocarcinoma of a rare histologic type and submitted a paper to the following journal: Modern Pathology 2019, 32: 269-279.
2. We participated in an international joint study of perivascular epithelioid cell tumors (PEComa), which are Uterine tumors of a rare histologic type, to clarify the relation of histologic findings to outcomes and submitted a paper to the following journal: Am J Surg Pathol 2018, 42: 1370-1383.
3. Continuing from last year, we performed a study with the Department of Obstetrics and Gynecology to examine the diagnostic accuracy and outcomes in patients in our hospital with endometrial carcinosarcoma. We found that endometrial carcinosarcoma can occur in young women and contains a mixture of well-differentiated and poorly differentiated components. We are preparing a paper.

Research on the respiratory organs
Depletion and clinical significance of the 3p22 region in the development of lung squamous cell carcinoma: The depletion of the short-arm region of chromosome 3 (3p) might occur most frequently in the earliest stage of development of lung squamous-cell carcinoma, suggesting that tumor suppressor genes exist in the 3p region. However, localized regions of candidate tumor suppressor genes related to carcinogenesis remain unclear, although at least 500 types of known genes exist in this 3p region. Microsatellite instability (MSI) analysis has been recognized as the most powerful method for determining the location of target genes by means of microsatellite makers near chromosomal genes. To determine the 3p region most strongly associated with the occurrence of lung squamous-cell carcinoma, we used a microdissection method to obtain cancerous and noncancerous tissues from surgically resected, unstained, formalin-fixed, paraffin-embedded samples from 81 patients with lung squamous-cell carcinoma. The DNA was extracted from these samples. A comprehensive MSI analysis was conducted with 18 markers in the 3p region. As a result, the MSI frequency of 18 markers in cancer tissues was 6% to 39% compared to non-cancerous tissues. Among 18 markers, MSI was most frequently found in the 3p22 region (39%). Therefore, we conclude that tumor suppressor genes related to the occurrence of lung squamous-cell carcinoma are likely in the 3p22 region.

Other studies
1. To determine age-related changes in the radius of the hepatic lobules, histometric analysis of liver tissue was performed in patients examined at autopsy. The radius (y) of the hepatic lobule increased with age (x). In patients 40 years or older, R2 calculated with the formula $y = 0.0032 x + 0.3167$ was approximately 0.65, indicating a strong correlation.
2. We have examined autopsy cases of fulminant group A streptococcal infection of the

right lower limb. At the sites of infection, many bacteria were present but inflammation was weak. Previous studies have demonstrated that fulminant group A streptococcal infection is associated with a weak or absent inflammatory response. In the present study, we did not investigate M proteins. However, previous studies have found that the inflammatory response is inhibited by such factors as hyaluronic acid capsules, M proteins, C5a peptidase, and nuclease.

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Department of Virology

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General Summary

Human herpesvirus (HHV) is frequently reactivated and can establish a lifelong latent infection of its host. We are studying the molecular mechanism of latency and the pathogenesis of human cytomegalovirus and HHV-6, and have found a novel latent protein of HHV-6 which is associated with ~~and~~ mood disorders. We are also applying HHV-6 and HHV-7 as tools for studying the mechanism of fatigue. The salivary levels of HHV-6 and HHV-7 DNA increase with training and decrease with rest, suggesting their usefulness as biomarkers of physiological fatigue and cancer-related fatigue (CRF).

Research Activities

Attenuation of HHV-6B reactivation by aging

Objective: Little research has examined HHV-6B in healthy adults, and the prevalence of HHV-6B in different age groups is unclear. Therefore, this study evaluated the seroprevalence of anti-HHV-6 antibodies in office workers and examined the effect of aging on seroprevalence. Because HHV-6B is reactivated in saliva, this study also investigated the association of age and HHV-6B reactivation by measuring salivary HHV-6 DNA levels.

Methods: The subjects were 77 office workers who underwent a health checkup. Anti-HHV-6 antibody titers were measured via enzyme-linked immunosorbent assay and salivary HHV-6 DNA levels. Associations were examined with age and, as confounding factors, with body mass index, smoking habit, and alcohol consumption.

Results: The seropositivity of anti-HHV-6 antibodies was significantly decreased in subjects 50 years or older, and anti-HHV-6 antibody titers were negatively correlated with age. Salivary HHV-6 DNA levels were also negatively correlated with age but were not significantly correlated with other factors.

Conclusions: Our results suggest that HHV-6B reactivation is attenuated by aging. Thus, anti-HHV-6 antibodies steadily decrease in the body with aging.

Clinical significance of CRF in patients with multiple myeloma

An adverse event in patients who have multiple myeloma (MM) and are treated with cytotoxic agents, proteasome inhibitors, and immunomodulatory drugs, such as bortezomib, lenalidomide, and thalidomide, is CRF. This study prospectively analyzed the clinical significance of CRF and evaluated the cumulative incidence of CRF and the survival rates of 16 patients who had MM and were treated with proteasome inhibitors and immunomodulatory drugs. Reactivation of salivary HHV-6 and HHV-7 was analyzed with the real-time quantitative polymerase chain reaction. The incidence of CRF was evaluated with a visual analog scale. The subjects were 11 patients with newly diagnosed MM and 5 with relapsed or refractory MM. The cumulative incidence of CRF was 54.9% overall

but was not associated with the type of treatment. The cumulative incidence of reactivation was 73.1% for HHV-6 and 45.6%, for HHV-7. However, the reactivation of HHV-6 and HHV-7 was not related to CRF. The overall survival and progression-free survival in patients with newly diagnosed MM was significantly shorter for those with CRF than for those without CRF. In conclusion, CRF is a major symptom in patients with MM and predicts shorter overall survival and progression-free survival in patients with newly diagnosed MM.

Increased levels of interleukin 1β and basic fibroblast growth factor in cerebrospinal fluid during HHV-6B encephalitis

A member of the β herpesvirus subfamily is HHV-6, which is further subdivided into HHV-6A and HHV-6B. Exanthema subitum typically results in fever and rash but resolves spontaneously without further complications or illness. However, in rare cases, HHV-6B infection can lead to encephalitis and has major clinical implications. Immunodeficiency associated with clinical procedures, such as hematopoietic stem cell transplantation, has been reported as a factor in HHV-6B-induced encephalitis; however, in cases of primary HHV-6B infection without immunodeficiency, the factors responsible for disease onset remain elusive. Levels of interleukin (IL) 1β and basic fibroblast growth factor (bFGF) in cerebrospinal fluid were found to be higher in patients with HHV-6B encephalitis than in patients with non-HHV-6B-induced febrile seizures. In vitro expression of the HHV-6B gene in infected U373 astrocytes was enhanced by IL-1β during the initial phase of infection and by bFGF during the maintenance phase. These findings indicated that IL-1β and bFGF contribute to HHV-6B growth and the onset of encephalitis.

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Department of Bacteriology

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General Summary

We are studying bacterial pathogenesis and host immune responses to bacteria and fungi. In particular, we focus on elucidating the molecular mechanisms of biofilm formation by *Staphylococcus aureus* and the pathogenesis of enterohemorrhagic *Escherichia coli*. We also conduct research for finding molecular targets to prevent biofilm-associated infections and for developing new pneumococcal vaccines. Active collaborative research with several basic and clinical laboratories have been conducted.

Research Activities

Iron-dependent periplasmic oxidative burst-mediated cell death identified in Gram-negative bacteria

Bacterial cell death and dormancy are keys against microbial infections, but the details, including underlying mechanisms, are poorly understood. Here, we identified iron-dependent periplasmic oxidative burst-mediated cell death induced in stressed Gram-negative bacteria, such as *E. coli*, including enterohemorrhagic *E. coli* O157, depending on the activity of the RNA polymerase sigma factor σ^s. Remarkably, this type of cell death, which was induced in dormant persister cells of stressed bacteria exhibiting outer-membrane disintegrity and periplasmic redox imbalance, was blocked by inhibitors of ferroptosis, a newly identified type of cell death in mammalian cells, or by the expression of a H_2O_2-degrading enzyme catalase in the periplasm but not in the cytosol. Furthermore, we demonstrated that the dormant bacteria evaded cell death by catalase from a commensal bacterium *Pseudomonas aeruginosa* or meat; when administered with meat, dormant food-borne pathogen *E. coli* O157 caused fatal infections in mice. In addition, on the basis of identified physiological attributes, we successfully developed a method that isolates dormant pathogen cells from contaminated food sources by avoiding cell death. In conclusion, this study provides evidence of the novel stress response and cell death pathway in Gram-negative bacteria, including food-borne pathogens, which might affect public health.

Modification of virulence and survival in the E. coli evolution by prophage module pmoAB that regulates bacterial gene expression

In microbial evolution, bacteriophages make a great contribution. The contribution of virulence factors, such as toxins, transferred into bacteria from bacteriophages is direct and clear; however, the evolutionary change of bacterial characteristics via prophage-derived genes acting such as a trans-species regulator is poorly understood. Here, we show that the virulence and survival of *E. coli* were regulated by a prophage module (designated

pmoAB) that represses expression of *rpoS* encoding the bacterial RNA polymerase sigma factor σ^S and that was found in enterohemorrhagic *E. coli* O157, a deadly food-borne pathogen emerged from a less-virulent *E. coli*. Specifically, via *rpoS* repression, *pmoAB* decreased the expression of bacterial stress-response genes but simultaneously increased the expression of virulence genes, such as type V-secreted serine protease and type-III secretion systems encoded within the virulence plasmid pO157 and exogenous elements in the bacterial genome, respectively. Under the control of *pmoAB*, *E. coli* O157 caused a drastically fatal infection in mice, and whereas, underwent high cell death via a periplasmic oxidative burst in dormant persister cells induced by stress-like *rpoS* mutants, suggestive of collateral damage. In conclusion, this study provides novel insights into microbial ecology and evolution, including bacteria-prophage interactions, and pathogenicity and survival, which might have implications for public health and food safety.

Role of gut microbe on host nitrogen cycle

Like oxygen, hydrogen, and carbon, nitrogen is an important element for the growth, maintenance, and survival of organisms. Nitrogen is abundantly present on Earth; however, it predominantly exists in the air as molecular nitrogen, which is inactive and cannot be used by organisms. Compared with the amount of the bioavailable forms of other elements, the amount of bioavailable nitrogen can often be insufficient, and this insufficiency can restrict the increase in the biomass of organisms. We investigated the roles of gut microbes on the nitrogen cycle in hosts.

Roles of lytic transglycosylases in biofilm formation and β-lactam resistance in methicillin-resistant S. aureus

While the functions of lytic transglycosylases in relation to cell division, biofilm formation, and antibiotic-resistance have been determined for several bacteria, their roles in *S. aureus* remain largely unknown. The only lytic transglycosylases known to occur in *S. aureus* are immunodominant staphylococcal antigen A (IsaA) and *Staphylococcus epidermidis* D protein. Our study demonstrated that, in a strain of methicillin-resistant *S. aureus*, IsaA and *S. epidermidis* D protein contribute differently to biofilm formation and β-lactam resistance. Our results suggest that IsaA is a potential therapeutic target for methicillin-resistant *S. aureus* infections.

Virulence of staphylococcal biofilm-dispersed cells

The biofilm dispersal process is the final stage of biofilm development and a necessary step for bacteria to leave the biofilm and spread in new locations. We have found that *S. aureus* causes biofilm dispersal by nuclease. We investigated the virulence of dispersed bacteria in comparison to planktonic bacteria in vitro and in vivo. Dispersed bacteria decreased phagocytosis by polymorphonuclear neutrophils. In a mouse model of infection, dispersed bacteria caused a lethal infection within 24 hours, but planktonic bacteria did not. These results indicate that dispersed bacteria from biofilm show higher virulence than do planktonic bacteria.

Basic research against biofilm formation of S. aureus

We found new insights of *S. aureus* biofilms. First, we demonstrated that RNA is a new component in biofilms. The RNA localized in the biofilm by binding to polysaccharides. Using several synthetic RNAs, we found several conditions of sequences to promote biofilm formation. Second, we demonstrated biofilm biomass is complemented by the cell wall anchoring protein *S. aureus* surface protein G and secreted extracellular adherence protein. Alternatively, these proteins might play different roles in the formation of the steric structure. Third, *Bacillus subtilis natto* inhibits the growth of *S. aureus*. Transcriptome analysis suggests that sporulation is important for this effect.

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Department of Tropical Medicine

Hirotaka Kanuka, *Professor*

Kenji Ishiwata, *Professor*

General Summary

Because of the failures of current eradication approaches and the logistical difficulties of implementing them, novel parasite control strategies need to be developed. An interesting aspect of parasitic diseases is that the vector arthropods that transmit the pathogens can mount immune responses against the infection that will kill a large proportion of parasites. Our group is pursuing research that covers 4 topics: (1) vector–parasite interactions, (2) infection responses in intermediate hosts, (3) immune responses to helminth infection, and (4) vector epidemiology.

Research Activities

Dissection of blood sucking behavior of mosquitoes

Exploring the molecular mechanism of the blood-sucking behavior of female mosquitoes is a critical step for fighting against vector-borne diseases, such as dengue and malaria, because pathogens are transmitted when mosquitoes are gorging on blood. To dissect the molecular mechanism of blood-sucking behavior, we performed RNA sequencing analysis and compared the expression pattern of genes in dengue and Zika virus-vector mosquito (*Aedes aegypti*) brains before and after blood suction. With this analysis, we noticed that the expression level of transient receptor potential-like (TRPL) was increased immediately after blood taking. With the CRISPR/Cas9 system, we have generated *TRPL* mutant mosquitoes. We found that camphor can directly activate TRPL in cultural cells. Furthermore, mosquitoes did not take camphor via membrane feeding when camphor was mixed with a phagostimulant. With these results, we hypothesize that mosquitoes stop sucking blood when they sense a camphor-like molecule in blood by TRPL, the expression of which is increased during the course of engorgement. We will investigate the blood/camphor taking behavior of *TRPL* mutants and verify our hypothesis.

Dissecting molecular interaction between entomopathogenic fungi and mosquito

Entomopathogenic fungi can invade a wide range of insect hosts in terrestrial and aquatic habitats and cause severe damage to insects. Upon fungal invasion, the innate immune system of insects mounts a response recruiting 2 distinct but complimentary forms, humoral and cellular immunity. Studies of insect-fungi interactions provide valuable insights into the coevolutionary arms race between entomopathogens and their hosts. In this study, to unveil fungi-insect molecular interactions, we performed biochemical characterization of virulence factors from *Beauveria bassiana*, a major entomopathogenic fungus capable of directly penetrating the cuticle of haematophagous insect hosts, the *Anopheles* and *Aedes* mosquitoes. Strains of *B. bassiana* were isolated from wild adult mosquitoes collected in Japan and West Africa, after which their pathogenicity against

laboratory-reared mosquitos (*A. aegypti*) was evaluated. Adult mosquitos infected with the *B. bassiana* 60-2 strain (B60-2) exhibited severe lethality, whereas mosquitos infected with the B9-3-1 strain showed less of an effect on their survival rate. Culture supernatants of B60-2, but not B9-3-1, showed more toxicity toward the adult mosquitoes when grown in the modified medium. To identify factors that can determine the different pathogeneses between these 2 strains of *B. bassiana*, the secreted proteins in the culture supernatant were compared, resulting in 2 proteins in the B60-2 culture supernatant being identified as candidate virulence factors. These results provide more insights into the ecological state of entomopathogenic fungus-mosquito interactions, which might imply an effective management of mosquito-borne diseases.

Toward the establishment of a more suitable strain of Lucilia sericata for maggot debridement therapy

Maggot debridement therapy (MDT) is an effective method for debriding wounds, such as leg ulcers, supporting wound bed preparation. The larvae of the blowfly species *Lucilia sericata* are the most widely used for MDT owing to their preference for feeding on necrotic rather than healthy tissue. New evidence suggests that maggots might contribute to wound healing in other ways, such as reducing biofilms, disinfecting wounds, and stimulating the growth of healthy tissue. In this study, several new strains of *L. sericata* were screened and established toward developing a maggot strain for more efficient MDT. One corpse-borne strain (strain 28) showed outcomes most favorable than did the conventional *L. sericata* strain for MDT in all 3 evaluation points: food consumptions, body weight, and growth rate. Strain 28 then underwent differential gene expression analysis via RNA sequencing. Compared to the genes of the conventional *L. sericata* strain, 1,623 genes of strain 28 showed higher expression and 1,370 genes showed lower expression. These findings indicate that strain 28 and the conventional strain hold unique gene expression patterns, which are likely related to the capacity of debridement and the potency of wound healing of the respective strains. The findings also suggest that new *L. sericata* strains might be developed for more efficient MDT by modifying these candidate genes.

Novel immune-mediated protection against reinfection of a gastrointestinal nematode causing chronic infection in mice

Heligmosomoides polygyrus, a murine gastrointestinal nematode, establishes in the small intestine of mice for more than 2 months. Orally inoculated infective larvae grow up in the muscular layer of the small intestine and emerge to the gut lumen by day 8 postinoculation. Secondary inoculation, performed at 4 weeks after deworming of the initial inoculation, terminates within 2 weeks after the inoculation making this parasite used as a reinfection model. Comparison of the number of larvae in the muscular layer between primary and secondary inoculation suggested an immune-mediated blockade against larval invasion into mucosal tissue. To confirm this possibility, the early phase of infection kinetics was evaluated. Results showed that the larvae first invade the stomach tissue and re-invade in the upper 1/6 part of the small intestine from days 1 to 2 postinoculation. The recovered number of larvae from the stomach between primary and secondary inoculation on day 1 postinoculation did not differ. However, the number of recovered larvae

from the same part of the small intestine differed between the 2 inoculations on day 2 postinoculation; a significant difference was found on day 6 postinoculation. These findings indicate that the first invasion of larvae into the stomach evokes a memory immune response that blocks the following invasion of the larvae into the upper part of the small intestine during days 1 to 2 postinoculation. Although the species specificity of blockade remains to be examined, this novel mucosal defense would open a new aspect of protective immunity.

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Department of Public Health and Environmental Medicine

Hiroyuki Yanagisawa, *Professor and Chairperson*
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Machi Suka, *Professor*
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General Summary

Our major research projects in the 2018 academic year focused on: (1) the effects of nanomaterials on chromosomal abnormality, (2) the effects of zinc-deficiency on the expression of interleukins associated with a decrease in anti-inflammatory M2 macrophages, (3) the disruption of prostaglandin synthesis pathway by environmental chemicals and related kidney abnormality, (4) molecular approaches toward cancer chemoprevention with food factors, (5) decompression stress in the hyperbaric work, (6) help-seeking intentions for mental illness, (7) postprandial hyperglycemia on the incidence of cardiovascular events and all-cause mortality in patients with type 2 diabetes, (8) job-related stress and health, and (9) the effects of polaprezinc, a carnosine-zinc complex, on pica and polydipsia or binge eating.

Research Activities

Experimental medicine

1. Mechanism of renal prostaglandin production via aryl hydrocarbon receptor

Aryl hydrocarbon receptor (AhR), a sensor protein activated by exogenous ligands, causes overproduction of prostaglandin E_2 and the onset of hydronephrosis in neonatal mammals. The activation of AhR elicits nuclear translocation, transcription enhancement, and activation of cytosolic signaling molecules, such as cytosolic phospholipase $A_2\alpha$. Using a mutant AhR lacking a nuclear translocation signal, we found that AhR nuclear translocation is required for transcription enhancement, cytosolic phospholipase $A_2\alpha$ activation, and prostaglandin E_2 production. These findings suggest a hitherto unknown cross-talk of transactivation activity in the nucleus and cytoplasmic signaling upon AhR activation.

2. Decompression stress in hyperbaric work

Exposure to a hyperbaric environment and the subsequent decompression of the surface might cause decompression stress. The level of decompression stress would be related to the risk of decompression sickness. Bubbles in the body after decompression and the level of human herpesvirus 6 in saliva might permit use of such biomarkers for research on decompression stress.

3. Role of macrophage subtypes in thymus atrophy of zinc-deficient rats and effects of interleukin 4 administration

Zinc deficiency causes growth retardation and dysfunction of the immune and reproductive systems. In this study, a relationship between thymus atrophy and zinc-deficiency was examined by the administration of interleukin 4 or by a change from a zinc-deficient diet to a zinc-standard diet. We found that the number of CD4 and CD8 double-positive

cells was significantly lower in rats fed the zinc-deficient diet than that in rats fed the zinc-standard diet. In contrast, the numbers of CD4 and CD8 double-negative cells, apoptotic cells, and macrophage subtypes (M1 and M2) were significantly higher in rats fed the zinc-deficient diet group than in rats fed the zinc-standard diet. These findings suggest that interleukin 4 administration results in a decreased inflammatory response and, therefore, in a decrease in M1 macrophages. The M2 macrophages remain elevated in this scenario, and we propose that they mediate a repair function.

4. Effects of nanomaterials on chromosomal abnormality in Chinese hamster lung cells

We examined the induction of micronuclei by exposure of AlO_2 or CeO_2 nanoparticles in both metabolic activation and inactivation in Chinese hamster lung cells. We also studied extracellular vesicles (EVs) secreted from human keratinocytes exposed to ZnO nanoparticles. The EVs were isolated from a culturing supernatant by means of ultracentrifugation. Dynamic light scattering analysis showed that the particle size of EVs was smaller from cells exposed to ZnO nanoparticles than from control cells. Sodium dodecylsulfate-polyacrylamide gel electrophoresis showed that several specific bands were contained in preparations of EVs from cells treated with ZnO nanoparticles. Subsequently, several types of keratin (such as keratin 1/2/5/6B/9/10) were detected with mass spectrometry.

5. Molecular approaches toward cancer chemoprevention with food factors

We have attempted to establish an evidence-based cancer prevention method with food factors. We reported that instant coffee powders inhibit the growth of human colon cancer HT-29 cells via cell-cycle arrest at the G2/M phase with up-regulation of cyclin-dependent kinase inhibitor p21.

Epidemiological studies

1. Development of persuasive messages encouraging help-seeking for depression

A cross-sectional Internet-based survey was conducted among Japanese adults to compare the responses to differently framed and formatted persuasive messages to encourage help-seeking for depression. Message framing and formatting might affect emotional responses to the message, the willingness to read the message, and the intention to seek help for depression. The application of loss framing and formatting to depression help-seeking messages would be recommendable.

2. Long working hours, sleep-related problems, and near-misses/injuries

We examined, in a nationally representative sample of workers, the association of long working hours/sleep-related problems and near-misses/injuries in industrial settings. Protective measures for workers, such as reducing the total hours of service/job-related fatigue, might effectively prevent near-misses/injuries in industrial settings.

3. Effects of polaprezinc on pica

Zinc deficiency has been associated with pica, although the role of zinc supplementation in the treatment of pica has not been well investigated. Polaprezinc is a complex of zinc and L-carnosine, both of which participate in the regulation of feeding behavior. We performed an open-label trial to evaluate the effects of polaprezinc on pica.

4. Effects of polaprezinc on binge eating

We performed an open-label trial to evaluate the effects of polaprezinc on binge eating and its related psychopathology in patients with a binge-eating disorder or bulimia ner-

vosa.

5. Optimal cutoff values of fasting plasma glucose variability for detecting retinopathy in type 2 diabetes

Our longitudinal study of 27 years provides new cutoff values for fasting plasma glucose variability. The threshold of fasting plasma glucose levels (6.9 mmol/L) is proposed to predict the incidence of retinopathy during the subsequent 25 years.

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Department of Forensic Medicine

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General Summary

Our main research projects in 2018 have focused on forensic pathology, DNA analysis, and forensic toxicology, as they have in the past. Much of the research was based on forensic practices. The details of our research are described below.

Research Activities

Forensic Pathology

1. Values of acrolein and several markers of patients dying in bathtubs

In Japan, many people die in bathtubs, where transient ischemic attack is believed to be contributory. We determined the values of protein-conjugated acrolein, polyamine oxidases (such as spermine oxidase and acetylpolyamine oxidase), and several markers in 10 cases of death in bathtubs and 10 control cases examined at forensic autopsies. We found that the values of these variables did not differ significantly between the groups. This lack of difference might be due to transient ischemic attack not playing a role in bathtub deaths, the low number of cases, and the values undergoing postmortem change. Therefore, future studies should involve a greater number of cases and the intergradation of each value due to the time since death.

DNA analysis

1. Identification of war-dead remains with DNA analysis

We performed identification of war-dead remains that had been recovered and repatriated from the former Soviet Union and areas further south by means of DNA analysis as part of the war-dead remains return project of the Ministry of Health, Labour and Welfare. For genetic markers, we used single nucleotide polymorphisms of hypervariable region of mitochondrial DNA and short tandem repeats (STRs) of nuclear DNA.

2. The detection and analysis of X chromosome STR loci

The analysis of STRs of the X chromosome is useful in kinship testing. We performed a detection and population genetic study of a novel tetranucleotide X chromosome STR (X-STR) locus. We analyzed the sequence structure of novel X-STRs, the appearance frequency of alleles, and forensic statistics data. We registered this data with the International Nucleotide Sequence Database Collaboration. We are planning to investigate the relevance with other X-STRs by linkage analysis.

3. Human height prediction by forensic DNA phenotyping

We examined h height prediction of human by forensic DNA phenotyping. Our analysis of human height and 16 single nucleotide polymorphisms (SNPs) showed a weak correlation. To accurately predict human height, the number of the smallest SNP should be used.

After having analyzed more SNPs, we believe that the SNP should be chosen with the contribution ratio being considered.

Forensic toxicology

1. Medicines and poisonous substances (abused drugs, alcohol, carbon monoxide, cyanide, and agricultural chemicals) suspected to have caused deaths were quantitatively analyzed with gas chromatography, gas chromatography/mass spectrometry, liquid chromatography–tandem mass spectrometry, and spectrum photometry in tissue specimens obtained at autopsy. Fluoride was quantitatively analyzed with the standard addition method.
2. We have constructed methods for drug screening using liquid chromatography–tandem mass spectrometry. Approximately 280 types of drug are targeted, but more target drugs should be added.
3. Analysis of fluoride was performed with gas chromatography–mass spectrometry for a forensic autopsy of a deceased person who was suspected to have consumed hydrofluoric acid. Fluoride was detected in the femoral vein blood. Therefore, the consumption of hydrofluoric acid was confirmed.

Radiocarbon analysis

1. Establishment of date of birth

We studied the estimation of date of birth from levels of carbon-14 isolated from the enamel or dentin of teeth. This method was used for postmortem examination, and its usefulness and problems were discussed. We also examined the effect of dental caries on the carbon-14 level. To apply this method to forensic medicine, we have examined the minimum amounts of enamel and dentin required for the analysis.

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Research Activities

Alimentary Tract

1. Examination of new biomarkers to assess disease activity in inflammatory bowel disease

(1) Prostaglandin E-major urinary metabolite as a reliable surrogate marker for mucosal inflammation in ulcerative colitis

We evaluated whether prostaglandin E-major urinary metabolite (PGE-MUM) can be used as a biomarker for ulcerative colitis (UC). Areas under the receiver operating characteristic curves of a simple clinical colitis activity index, the Mayo Endoscopic Score, and the Matts' grade (histologic activity) were each higher for PEG-MUM than for C-reactive protein.

The main advantage of PGE-MUM appears to be the differentiation of colonoscopic or histologic remission from active disease in UC. On the other hand, this marker was lower in patients with UC in remission than in healthy volunteer subjects. Compared with the C-reactive protein level, the PGE-MUM level demonstrated greater sensitivity for reflecting UC activity, especially in cases of histologic inflammation, and, thus, seems to be a better evaluator of mucosal healing.

On the basis of this result, we have performed a comparative trial to identify the most reliable marker among the immunochemical fecal occult blood test, fecal calprotectin, and PGE-MUM for detecting endoscopic mucosal healing in patients with UC.

(2) The clinical benefit of serum and tissue interleukin 6 to assess disease activity and severity in inflammatory bowel disease

Levels of serum interleukin 6 are relevant to UC, Crohn's disease, and systemic inflammatory responses. Herein, we hypothesize that serum and tissue levels of interleukin 6 are helpful for predicting treatment efficacy in UC and Crohn's disease, especially for the evaluation of anti-tumor necrosis factor (TNF) α antibody treatment.

2. Examination of the efficacy of anti-TNF-α antibodies for treating intestinal Behçet's disease

We reviewed the clinical background, effects, and outcome of treatment with an anti-TNF-α antibody for intestinal Behçet's disease at our hospital. The anti-TNF-α antibody was administered to 9 patients, who had been previously treated with infliximab (6 patients), adalimumab (2 patients), and infliximab followed by adalimumab (1 patient). All patients had ulcers surrounding the ileocecal region; treatment with prednisolone was

ineffective in all patients, but a secondary treatment with infliximab was effective in 6 of 7 patients. Treatment with infliximab eventually became secondarily ineffective in 5 patients, but 2 of the 2 other patients responded to treatment with adalimumab and were able to remain in remission.

3. The development of molecular-specific cancer theranostics

We have developed a method for molecular-specific phototherapy based on near-infrared fluorescence molecular imaging.

4. Examination of characteristics of primary gastrointestinal malignant lymphoma

Primary gastrointestinal malignant lymphomas account for 3% to 4% of extranodal lymphomas. We reviewed the clinical features (histotype, localization, and stage) of 166 cases that were histologically diagnosed as primary gastrointestinal malignant lymphoma in our hospital from October 2007 through September 2017. The types of lymphoma identified in these cases were mucosa-associated lymphoid tissue lymphoma, 49.4%; diffuse large B-cell lymphoma, 28.9%; follicular lymphoma, 14.5%; mantle cell lymphoma, 3.0%; Burkitt's lymphoma, 1.8%; and T-cell lymphoma, 2.4%. Twenty-one cases (13%) had lesions in multiple organs of the digestive tract, and duplication rate was significantly higher for mantle cell lymphoma, follicular lymphoma, diffuse large B-cell lymphoma than for mucosa-associated lymphoid tissue. In the case of these tumors, careful examination of the entire digestive tract is necessary.

5. Examination of biological malignancy analyzed from the rate of vascular invasion positivity in resected gastrointestinal tract neuroendocrine tumors

Gastrointestinal neuroendocrine tumors and neuroendocrine cancers are rare, but the number of patients is increasing. Gastrointestinal neuroendocrine tumors or neuroendocrine cancer was diagnosed in 144 patients (156 lesions), who underwent endoscopic or surgical resection in our hospital from February 2008 through October 2017. The degree of malignancy and the presence of vascular invasion and distant metastasis were examined, and the presence or absence of vascular invasion according to the tumor diameter was analyzed. The lesion was most often in the rectum (65.4%), followed by the duodenum (16.7%), the stomach (12.8%), the appendix) (3.2%), the colon (1.3%), and the jejunum and ileum (0.6%). The treatments were 71.8% for endoscopic resection, 18.6% for surgical resection, 57.1% for Grade 1 (G1) neuroendocrine tumors, 8.3% for G2, 0.6% for G3, and others unknown. Vascular invasion occurred at a high rate, even for G1 neuroendocrine tumors with a diameter less than 10 mm, and included venous infiltration (15.6%) and lymphatic vessel infiltration (7.8%).

6. Comparison of endoscopic hemostasis and percutaneous arterial embolization re-bleeding rate for colonic diverticulum hemorrhage and retrospective examination of risk factors for re-bleeding

We compared the results of emergency endoscopy and percutaneous arterial embolization with interventional radiology (IVR) in 207 patients hospitalized for diverticulum hemorrhage from November 2012 through December 2017 and examined risk factors for re-bleeding. Of the 207 cases of diverticulum hemorrhage, 93 cases with massive hemorrhage were subjected to abdominal enhanced dynamic computed tomographic imaging, 34 cases showed active extravasation, and 14 cases were conducted prior to IVR. Endoscopy was performed prior to IVR in 193 of 207 cases, and IVR was also performed owing

to hemostasis difficulties in 12 cases. Re-bleeding was observed in 1 (3.8%) of 26 patients who underwent IVR and in 27 (14.8%) of 181 patients who underwent hemostasis or were follow-up with endoscopy alone. Risk factors for re-bleeding included hypertension, diabetes, renal failure, smoking history, and the administration of nonsteroidal anti-inflammatory, antithrombotic, or anticoagulant drugs; however, multivariate analysis showed that hypertension ($p = 0.0423$) and smoking ($p = 0.0486$) were independent risk factors.

7. Examination of irregular change of gastric mucosa newly generated after *Helicobacter pylori* eradication

After *H. pylori* is eradicated, although the development of gastric cancer is suppressed, irregular changes are generated in some cases, which makes the endoscopic diagnosis of gastric cancer difficult. From 2005 through 2017, 352 cases who received eradication therapy were reviewed, in which the eradication history was clear and gastric mucosal changes before and after eradication were able to be analyzed temporally and histologically with endoscopic images. Temporal changes in gastric mucosa after *H. pylori* eradication were classified into 3 groups based on the ease of endoscopic observation: Easy group (175 of 352 cases, 49.7%), Invariant group (87 of 352 cases, 24.7%), and Difficult group (90 of 352 cases, 25.6%). The groups did not differ significantly in patient age, sex, or gastric mucosal atrophy before *H. pylori* eradication. An increase of crypt epithelia was observed in 51.9% of cases, which is the main reason for the Difficult group, in the process of eliminating inflammation after *H. pylori* eradication and the mechanisms of repair and regeneration. On the basis of these results, we believe that gastric cancers will be more difficult to detect after eradication than before eradication in approximately one-fourth of the stomach and that careful endoscopy, similar to that of an infected stomach, is required.

8. Nutrition treatment for inflammatory bowel disease

We have found that an omega 3 diet, in which omega-3 polyunsaturated fatty acids are consumed, can maintain the remission of inflammatory bowel disease (IBD).

9. Study of fatty acids in Crohn's disease

In patients with Crohn's disease, various fatty acids were specifically altered in both plasma and erythrocytes, and plasma palmitic acid and the plasma Crohn's disease fatty acid index are potentially useful as new serological markers for Crohn's disease.

10. The study of mutations of the genes nudix hydrolase 15 (NUDT15) and inosine triphosphatase (ITPA) in patients with IBD

Although mutations of both the nudix hydrolase 15 and inosine triphosphatase genes are strongly associated with myelosuppression due to azathioprine in Japanese patients with IBD, metabolite concentrations are not changed.

11. Examination of a smoking habit and intestinal flora in patients with IBD

The effects of smoking habits on patients with IBD were examined and found to alter the intestinal flora.

12. Examination of the efficacy of indigo naturalis (*qing-dai*) in ulcerative colitis

The administration of indigo naturalis (*qing dai*) for 2 weeks significantly improves the clinical activity of ulcerative colitis.

Liver

1. The development of targeting therapy for cancer stem cells in liver cancers

The only curative treatments for primary liver cancers are surgical resection at an early stage. However, most cancers are diagnosed at advanced stages, when extant therapies are ineffective. Therefore, novel molecules that can become targets for future therapies are urgently needed to be identified. We have reported that spalt-like transcription factor 4 (SALL4) regulates the cell fate decision in hepatic stem/progenitor cells during normal liver development, is indicative of aggressiveness and poor prognosis, and maintains the stemness of cancer stem cells in liver cancers. Further analyses of cancer stem cell-mediated mechanisms might provide a novel future therapeutic strategy against liver cancers.

2. Pathogenesis and messenger RNA and micro RNA expression profiling of primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH)

The pathogenesis of autoimmune liver disease such as PBC and AIH is unknown. To investigate the pathogenesis and identify novel therapeutic targets, we performed microarray analysis of messenger (m) RNA and micro (mi) RNA expression in CD4+ T cells derived from 14 patients with primary biliary cholangitis (PBC). We found decreased expression of 4 miRNAs (miR-425, -181a, -181b, and -374b), which dysregulate T-cell antigen receptor signaling in PBC-CD4 T+ cells. In particular, the decreased expression of miR-425 strongly induced inflammatory cytokines via N-Ras upregulation in the T-cell antigen receptor signaling pathway, suggesting that the restoration of decreased miR-425 or the suppression of N-Ras is a promising immunotherapeutic strategy against PBC.

3. The relationship of nutritional condition and neuropsychological test results in patients with liver cirrhosis

Cases of liver cirrhosis complicate minimum hepatic encephalopathy and have caused traffic accidents and communication problems. Neuropsychological disturbance is typical of minimum hepatic encephalopathy. However, the diagnostic criteria for minimum hepatic encephalopathy have not been clarified. We studied the pathophysiological findings of minimum hepatic encephalopathy using a neuropsychological test and a food frequency questionnaire. Of patients with liver cirrhosis, 17% had abnormal results of the Digit Symbol Test. Such abnormal results are related to the Child-Pugh score, the serum albumin level, the branched chain amino acid and tyrosine ratio, nutrient intake as usual energy intake, and the fat energy ratio. Thus, nutrition support using the food frequency questionnaire might be a useful method to prevent minimum hepatic encephalopathy.

4. Investigation of the Frailty index in elderly patients with digestive disease

We evaluated whether simplified Frailty scores are associated with clinical outcomes or adverse outcomes after treatment in patients 80 years or older with digestive disease.

5. Examination of the dynamics of blood cholesterol levels before and after treatment with sofosbuvir and ribavirin for hepatitis C virus genotype 2

In its life cycle, hepatitis C virus (HCV) uses and affects the host's lipid metabolism. Infection with HCV reduces the total cholesterol level, but the change varies in degree depending on genotype, can be improved by elimination of HCV, and is reversible.

We investigated blood lipid dynamics before and after treatment of HCV genotype 2 with sofosbuvir and ribavirin. Levels of total cholesterol and low-density lipoprotein choles-

terol did not change during the treatment period but increased significantly 4 weeks after the end of treatment.
On the other hand, levels of high-density lipoprotein cholesterol increased 4 weeks after the start of treatment and remained elevated for 24 weeks after the end of treatment. In the group of patients in whom the hemoglobin level at the end of treatment had decreased from the baseline level by 2 g/dl or more, the total cholesterol level decreased during treatment but recovered to the same level as in the group in which hemoglobin had not decreased 4 weeks after the end of treatment.
6. Study of characteristics of acute-onset autoimmune hepatitis presenting central lobular zone and band necrosis and its fine classification
Because acute-onset autoimmune hepatitis (AIH) may lead to acute liver failure, it should be promptly diagnosed and treated. Recently, unlike typical AIH with interface hepatitis, AIH that causes central lobular zone and band necrosis (CZN) in the central vein area has been reported, and its laboratory findings and immunogenetic factors have been shown to differ from those of typical AIH. Therefore, we examined the clinical features of acute-onset AIH exhibiting CZN and found that this type of AIH was frequently associated with inflammation in the portal region with low levels of alkaline phosphatase and γ guanosine triphosphate and with low levels of immunoglobulin G, immunoglobulin M, and total bilirubin. In addition, we found that the frequency of HLA-DR9 was higher in cases of acute AIH with CZN than in cases without CZN and that the frequency of HLA-DR 13 tended to be higher in pure AIH phenotype than in mixed AIH phenotypes.

Gall bladder and Pancreas
1. The mechanistic effects of cigarette smoking on the development of pancreatic cancer
Pancreatic cancer is extremely aggressive, and the results of even advanced medical treatment remain unsatisfactory. Smoking is a major risk factor for various malignancies, including pancreatic cancer. Clarifying the mechanism of how smoking enhances the development of pancreatic cancer can be a useful tool for decreasing its incidence and developing effective treatments. Therefore, we are studying the effects of smoking on the autophagy mechanism in patients who have pancreatic cancer.
2. Clinical trial of a surveillance-targeted intraductal papillary mucinous neoplasm with endoscopic ultrasonography for early detection of pancreatic cancer
In patients with an intraductal papillary mucinous neoplasm (IPMN), frequent are both the canceration of an IPMN and the coexistence of normal-type pancreatic ductal carcinoma. Furthermore, pancreatic cancer has an extremely poor prognosis, and detection of a tumor at a diameter of 10 mm or less is reportedly necessary to expect a long-term survival. Because endoscopic ultrasonography is the most sensitive method for detecting small pancreatic cancers, it is considered to be the main strategy for early diagnosis. However, because pancreatic cancer progresses rapidly and has no effective biomarkers for early detection, periodic surveillance under a high-risk group enclosure is needed for early diagnosis. We are examining whether periodic surveillance with endoscopic ultrasonography in patients with an IPMN contributes to early detection of pancreatic cancer. We are also attempting to establish a more effective and executable surveillance strategy.

3. Study of the effect of corticosteroid treatment on diabetes management for autoimmune pancreatitis

Autoimmune pancreatitis (AIP) is an immunoglobulin G4-related disease for which a corticosteroid (prednisolone) is effective for treating pancreatic lesions and whose onset involves autoimmune mechanisms. Generally, AIP frequently complicates diabetes mellitus, but because the effect of prednisolone treatment on the management of diabetes mellitus is unclear, the medium- and long-term effects on diabetes mellitus management were examined. Of the 28 cases of AIP at our hospital, 18 were associated with diabetes mellitus. We divided the cases into 3 groups on the basis of the time of diabetes mellitus onset — before prednisolone introduction (pre-existing group), at AIP diagnosis (new-onset group), and after prednisolone introduction (after prednisolone group) — and retrospectively analyzed changes of HbA1c values and the contents of diabetes mellitus treatment. Prednisolone was introduced in 14 cases (77.8%), and imaging findings and blood biochemical responses were obtained in all cases. The HbA1c value improved 1 month after prednisolone administration in 2 cases (14.3%), but improved after 12 months in 7 cases (50.0%). The patient's age when AIP developed in the group in which the HbA1c level improved after 12 months was lower than in the group without improvement. In the pre-existing group (n = 7), diabetes mellitus did not improve 24 months after prednisolone introduction. Treatment with diet alone was difficult in the pre-existing group, but diet alone was effective in the new-onset and post-prednisolone groups, and doses of insulin could be reduced. The patients in whom prednisolone was not introduced were older (72 ± 8.7 years old) than those in the prednisolone administration group (74 ± 9.2 years old).

4. Clinical analysis of long-term management of AIP

Whether administration of a low corticosteroid dosage must be continued to maintain remission of AIP remains controversial.

Considering the side effects of corticosteroids, the minimum dosage required to maintain remission and the predictors of recurrence due to corticosteroid withdrawal must be clarified. We analyze the clinical data of cases of AIP at our hospital and are aiming to construct a treatment strategy for long-term management.

5. Clinical research about the relationship of gut microbiota and oxidative stress with pancreatic cancer

Gut microbiota have received substantial attention as a research topic in various diseases, including malignant tumors. In particular, the effects of short-chain fatty acids (SCFAs) in several cancers have been reported. Oxidative stress, which is a risk factor of senescence, is also a recent subject of cancer research. However, the effects of SCFAs and oxidative stress in pancreatic cancer growth are still unknown. Therefore, we will assess the clinical usefulness of several SCFAs and oxidative stress markers as potential markers of diagnosis and prognosis in patients with pancreatic cancer.

6. The investigation of Wilm's tumor protein 1-pulsed dendritic cell vaccines for patients with advanced pancreatic cancer

Wilm's tumor protein 1-targeted cancer vaccine might be effective for treating patients who have pancreatic ductal adenocarcinoma.

Chemotherapy

1. Optimized and personalized treatments for elderly patients with cancer

New chemotherapy drugs have recently been developed, and considerable improvement was observed in the prognosis of advance and recurrent malignancies of the digestive system. Owing to the rapid aging of the population, many patients who have cancer are 80 years or older. The management of cancer in elderly patients is challenging because of the difficulty of assessing the benefits of treatment and the decreased tolerance of anticancer therapy. We analyzed the prognosis, adverse effects, and treatment regimen of elderly patients with cancer at our hospital and have developed optimized and personalized treatments.

2. Cancer associated venous thromboembolic disease and systemic chemotherapy

Venous thromboembolic (VTE) disease is often found in patients who have cancer. Such VTE disease is life-threatening and is the second most common cause of death in patients with cancer. Bleeding from the alimentary tract is frequently observed in patients who have malignancies in the digestive system; thus, the possibility of VTE disease should be evaluated in all patients, and the risks associated with anticoagulant therapy should be assessed. We analyzed the risk of VTE, the chemotherapy regimen, and the treatment of VTE in patients of our hospital and then optimized cancer and VTE treatment.

3. Search for biomarkers of the efficacy and safety of nivolumab for advanced and recurrent gastric cancer

Nivolumab is a newly developed immune checkpoint inhibitor that enhances antitumor activity through programmed death 1 receptor. Despite severe adverse reactions having occurred in 18% of patients treated with nivolumab, the response rate with nivolumab was only 11%. Considering the cost and benefits of nivolumab, biomarkers that predict the efficacy and safety of nivolumab are desired. We are working with National Cancer Center of Japan to achieve this aim.

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General Summary

Our research in 2018 consisted of the following areas: (1) cerebrovascular disease and (2) neurodegenerative disease. We have performed studies of clinical and basic neurology.

Research Activities

Cerebrovascular disease

First, we have played a leading role in a multicenter study to investigate the clinical characteristics of juvenile stroke and have taken part in a multicenter study to clarify the characteristics of patients with stroke who are taking oral anticoagulants. Second, we have worked to establish an intrahospital system to treat patients with hyperacute stroke, consisting of stroke coordinate nurses who support the hyperacute treatment, including thrombolysis, and a new smartphone application (JOIN®) that can share texts, neuroimages, photographs, and videos among the stroke team. By using this application, we can evaluate stroke volume and the site of occlusion with the same quality as with the monitor of a personal computer. Third, we have performed several prospective and retrospective studies from our stroke care unit registry.

The main themes of our clinical studies are as follows:

1) To survey the current status of diagnosing deep vein thrombosis (DVT) via ultrasonographic examination of leg veins for patients with acute stroke and DVT

2) Right-to-left shunt evaluation with a novel probe (pasteable soft ultrasound probe)

3) To investigate whether the JOIN® application would shorten the time from admission to thrombolysis and mechanical thrombectomy in patients with hyperacute ischemic stroke

4) To investigate the relationship of cerebral microbleeds and functional outcomes in patients with a noncardiogenic transient ischemic attack and minor ischemic stroke

5) To investigate the proportion and explored factors for patients who have acute ischemic stroke with a poor outcome 90 days after onset but a good outcome at hospital discharge

6) To investigate the prevalence of DVT and pulmonary embolism in patients with a cryptogenic stroke and to investigate the clinical features of cryptogenic stroke in relation to the location of a DVT

Neurodegenerative disease

1. Parkinson's disease

We have continued clinical studies of cardiovascular autonomic dysfunction in patients with Parkinson's disease (PD). We investigated the association of cardiovascular autonomic dysfunction and cognitive impairment in patients with PD. We also evaluated the effect of a dopamine agonist (rotigotine) on nocturnal hypotension observed with a 24-hour ambulatory blood pressure monitoring test. Second, we compared the correlation with assessments of dopamine transporter scan, myocardial ^{123}I-meta-iodobenzylguanidine scintigraphy, and orthostatic hypotension between depression and anhedonia in patients with drug-naive PD. Third, we investigated whether the characteristics of white and red blood cells are associated with clinical symptoms in patients with drug-naive PD. Fourth, we developed a simple smell identification test to aid the diagnosis of PD and compared this test with standardized olfactory tests for Japanese patients. Finally, we examined the patterns and trends in anti-PD drug prescriptions in 20,936 patients (aged ≥30 years with newly diagnosed PD) using nationwide registry data from 2008 through 2016.

2. Multiple system atrophy

We investigated the association of dysphagia and vocal cord palsy in multiple system atrophy to assess the optimum timing of percutaneous endoscopic gastrostomy.

Basic research

1. PD

Mutations of the retromer component vacuolar protein-sorting protein 35 is linked to autosomal dominant forms of familial PD, PARK17. We analyzed the movement and function of the retromer in neurons differentiated from induced pluripotent stem cells from patients with PARK17.

2. Amyotrophic lateral sclerosis

Reduced expression of the survival of motor neuron gene is suggested to be a risk factor for amyotrophic lateral sclerosis. We have investigated the role of survival of motor neuron protein in the pathogenesis of amyotrophic lateral sclerosis. We are also developing induced pluripotent stem cells introduced mutation in transactive response DNA-binding protein.

3. Peripheral neuropathy

We investigated the pathogenesis of hypothyroidism-related neuropathy. We generated a mouse model of adult-onset hypothyroidism and analyzed in these mice hormonal status, mechanical thresholds, electrophysiological properties, and voltage-gated potassium channel protein levels of sciatic nerves.

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Department of Internal Medicine
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General Summary

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

Research Activities

Studies of immunoglobulin A nephropathy
A multicenter, prospective cohort study (The Japan IgA Nephropathy Cohort Study) is currently in progress. The study will validate the effectiveness of a series of therapeutic regimens, including tonsillectomy or corticosteroid therapy or both, which is widely accepted in Japan. The post-hoc analysis of this retrospective multicenter large-scale study is in progress to validate therapies for advanced cases of immunoglobulin A nephropathy at the time of biopsy diagnosis.

Studies on total nephron number counting
We have performed stereology-based total nephron number (TNN) counting for kidneys obtained at autopsy from Japanese subjects. The study revealed that the TNN in Japanese subjects is one of the lowest nephron counts yet reported. Through the combined use of images and biopsy specimens, a study to examine TNN in clinically available settings is currently in progress.

Studies of CKD-mineral and bone disorder
We previously reported that the DNA methylation patterns in calcium sensing receptor (*Casr genes*) and vitamin D receptor (*VDR*) genes were modified in the parathyroid glands of CKD-mineral and bone disorder (MBD). We then analyze the effect of histone modification and the cell cycle in the parathyroid glands of CKD-MBD. Furthermore, we are investigating how glial cells missing 2 (gcm2 genes) in the parathyroid gland, which is the essential transcription factor for parathyroid development in terrestrial vertebrates, affects parathyroid gland function. Magnesium was associated with the suppression of vascular calcification and the mortality in patients undergoing hemodialysis (HD). On the

basis of these findings, we have shown in a prospective cohort study that proton pump inhibitor use is associated with an increased risk of hypomagnesemia in patients undergoing HD (PLOS One 2015). Furthermore, we examined the association of mortality with levels of serum total magnesium and ionized magnesium. Because glycometabolism is now attracting attention in various fields, we investigated insulin resistance in patients with CKD of stages 2 to 4. Our study clarified the association of insulin resistance and fibroblast growth factor 23 in patients with CKD. To clarify the association of insulin resistance with all-cause mortality, cardiovascular events, and CKD-MBD in patients receiving HD, we are now performing a cohort study.

Studies of peritoneal dialysis

We have reported that the prevalence of peritoneal dialysis (PD)-associated peritonitis and outcome, including patient survival and technical survival, did not differ significantly in patients receiving PD between those who have or do not have diabetes. We have reported that the lipid profile was associated with the deterioration of residual renal function in patients starting PD. We are conducting clinical research on a bicarbonate/lactate-buffered neutral PD solution, the clinical efficiency of incremental PD, and peritoneal membrane pathology. Additionally, we have started using a newer ultrafine laparoscope to evaluate peritoneal injury.

Renal protective effects of azilsartan in a rat model of adenine-induced renal failure

Although daily urinary sodium excretion was decreased in rats to which adenine was not induced, azilsartan suppressed the decreasing sodium excretion, urinary protein excretion, and sympathetic nerve activity. We found that a molecular mechanism of renal protection by azilsartan is the effect for natrium transporter.

Relationship between clinical character of primary aldosteronism and hormone kinetics of the renin-angiotensin system

To simplify the diagnosis of primary aldosteronism, we are evaluating the relationship between clinical character of primary aldosteronism and the results of several challenge tests or adrenal venous sampling.

Basic study of kidney regeneration: Regenerative potential of induced pluripotent stem cells

We generated induced pluripotent stem cells (iPSCs) from patients undergoing HD due to diabetes nephropathy and glomerulonephritis (HD-iPSCs) as representatives of CKD-iPSCs or from healthy control subjects (HC-iPSCs). The HD-iPSCs differentiated into nephron progenitor cells (NPCs) with similar efficiency to HC-iPSCs. Additionally, HD-iPSC-derived NPCs expressed comparable levels of NPC markers and differentiated into vascularized glomeruli upon transplantation into mice, as HC-iPSC-derived NPCs. Our results indicate the potential of HD-iPSCs as a source of cells for kidney regeneration.

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Department of Internal Medicine
Division of Rheumatology

Daitaro Kurosaka, *Professor* Ken Yoshida, *Assistant Professor*

General Summary

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

Research Activities

We have performed clinical and experimental studies of rheumatic diseases.

1. Fasciitis in dermatomyositis

We have previously demonstrated that fasciitis is a common lesion of dermatomyositis detectable early after disease onset with *en bloc* biopsy and magnetic resonance imaging. Therefore, the detection of fasciitis plays an important role in the diagnosis of dermatomyositis, especially at an early stage. Power Doppler ultrasonography is useful for detecting inflammation and vascularity in rheumatic diseases. We have shown that fasciitis is detected with power Doppler ultrasonography in patients with dermatomyositis and that angiogenesis is observed in fasciitis associated with dermatomyositis. This year, we examined with immunohistochemical staining whether angiogenesis-related factors and inflammatory cytokines are expressed in the fascia. We found that angiogenesis, the number of cells expressing vascular endothelial growth factor, and the number of cells expressing tumor necrosis factor α were higher in the fascia of dermatomyositis (DM) than polymyositis and were increased predominantly in the fascia rather than in the muscle of the early-phase DM. The degree of inflammation correlated with that of angiogenesis in the fascia of DM. We speculate that the fascia can, therefore, be a primary site of inflammation and angiogenesis in the pathogenesis of DM. We are conducting research with RNA sequencing analysis on gene expression in the fascia and muscle in patients with DM compared with polymyositis.

2. Neuropathic like pain in patients with rheumatoid arthritis

Pain in rheumatoid arthritis (RA) has been thought to be due to nociceptive pain, but it was recently reported to also include a mechanism associated with neuropathic pain. Therefore, we examined the frequency and clinical characteristics of patients who have RA and neuropathic-like pain. Neuropathic-like pain with RA was evaluated with the PainDETECT Questionnaire, a screening tool for neuropathic pain. We compared the clinical variables between patients with and without neuropathic-like pain. We showed that neuropathic-like pain in patients with RA was associated with subjective indicators, including tender joint count and the health-related quality of life, rather than with objective indicators of disease activity, including swollen joint count C-reactive protein, and the erythrocyte sedimentation rate. Proper treatment of neuropathic-like pain in patients

with RA might improve the health-related quality of life. This year, to examine central sensitization in patients with RA we have used the central sensitization inventory and analyzed the change of the central nervous system in a mouse model of RA.

3. Citrullination of peptidylarginine deiminase in RA

Citrullination, catalyzed by peptidylarginine deiminase (PAD), is a posttranslational modification of arginine to citrulline, which contributes to the pathogenesis of RA. We performed a study to examine the presence and functions of citrullinated chemokines in RA. A newly developed enzyme-linked immunosorbent assay system showed that concentrations of citrullinated epithelial-derived neutrophil-activating peptide 78 (ENA-78)/chemokine (C-X-C motif) ligand 5 (CXCL5) in synovial fluid were higher from patients with RA than from patients with other rheumatic diseases and correlated with the C-reactive protein level and the erythrocyte sedimentation rate. Although ENA-78/CXCL5 is a neutrophil chemotactic factor, an in-vitro chemotaxis assay and in-vivo experiments showed that citrullinated ENA-78/CXCL5 has a monocyte-recruiting function and stimulates inflammation in a model of inflammatory arthritis. Recently, autocitrullination of PAD has also been reported. In general, the enzyme activity of PAD is decreased after citrullination. However, the function of citrullinated PAD other than enzyme activity remains unclear. Last year, we found that citrullinated recombinant human PAD had monocyte-chemotactic activity in vitro and arthritis-inducible activity in vivo, whereas noncitrullinated PAD did not. We are trying to develop a detection system for citrullinated PAD in patients with RA.

4. *Bombina variegata* peptide 8/prokineticin 2 in RA

Prokineticin and its receptors are expressed in various tissues and are involved in diverse physiological functions, such as angiogenesis, neurogenesis, circadian rhythm, and the pain threshold. Of these functions, angiogenesis plays an important role in the pathogenesis of RA. We previously investigated the expression of prokineticin 2 and its receptors (prokineitcin receptor 1 and prokineticin receptor 2) in mice with collagen-induced arthritis, the animal model of RA, and reported that the expression levels of prokineticin 2 and prokineticin receptor 2 are significantly elevated in the joints of collagen-induced arthritis mice and correlate with the severity of arthritis. Therefore, we investigated the effect of an antagonist of prokineticin 2 on collagen-induced arthritis. Our data showed that administration of a prokineticin 2 antagonist suppresses the severity of arthritis. However, whether the effect of this antagonist depends on prokinteicin receptor 1 or prokinteticin receptor 2 is unclear. This year, we attempted to establish a tissue-specific prokineticin receptor 2 knockout mouse and have established a prokineticin receptor 2 floxed mouse.

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Department of Internal Medicine
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General Summary

We have 6 research groups across the broad field of cardiology. In these study groups, we have been studying the problems that face us in clinical practice. Our research is based on clinical studies that use a large database we have been developing. Specifically, we have recently used covariance structure analysis as a new solution for action assignments. Basic research is also performed to solve clinical questions.

Research Activities

Ischemic Heart Disease Research Group
Through examination and treatment, we are creating a database of all patients and are researching the relationship of risk factors for ischemic heart disease, treatment, prognosis, and other factors and hope to publish our findings. In addition, transcatheter aortic valve implantation was started in 2015, and we are investigating for our own data. We are giving presentations at all conferences we attend, including those overseas.
We are focusing on performing physiological examinations to evaluate myocardial ischemia. In particular, we evaluate functional ischemia by measuring the fractional flow reserve, the instantaneous wave-free ratio, and the resting full-cycle ratio. We are researching a prognostic evaluation and the relationship with many factors through physiological results. In percutaneous coronary intervention, the placement of drug eluting stents (DESs) is now mainstream. Considering the long-term results of and research on each DES, we are selecting appropriate DESs. Through the use of imaging devices, such as intravascular ultrasound and optical coherence tomography/optical frequency domain imaging, we are improving treatment results and clarifying the pathogenesis of coronary artery disease. Optical coherence tomography, angiosynchronization, and instantaneous wave-free ratio angiosynchronization have recently been available, and they would be useful methods for further treatment improvement. We are also participating in ongoing multi-institutional research studies and are contributing to the creation of new evidence by participating in national-scale clinical research at our hospital.

Arrhythmia Research Group
We are conducting clinical research based on electrophysiological examination for all

supraventricular and ventricular arrhythmias. In clinical practice, atrial fibrillation (AF) accounts for the majority of arrhythmias; therefore, our main research focuses on AF. Although catheter ablation is now a curative therapy for AF, its safety and success rate are still insufficient; therefore, we provide new findings at home and abroad by conducting various clinical research studies.

With regard to paroxysmal AF, several balloon technologies, including the cryoballoon, hot balloon, and laser balloon, have been emerging, and a high success rate, equivalent to that of radiofrequency ablation, has been reported. However, the data regarding complications (such as pulmonary vein stenosis, phrenic nerve and esophagus injury, and asymptomatic cerebral infarction) and long-term outcomes after catheter ablation according to different ablation methods are still limited. In addition, we have investigated the clinical and procedural factors associated with AF recurrence and complications to clarify the optimal treatment for each patient. On the other hand, ablation therapy for persistent and chronic AF has not been established. We aim to clarify the mechanisms of AF with various mapping systems and to evaluate and compare the therapeutic effects by modifying AF substrates among various ablation strategies.

In our team, catheter ablation therapy for arrhythmias has been the main subject, and we always aim to provide patients with the best treatment by adopting the novel therapies and investigating various clinical data.

Heart Failure Research Group

1. Study group on heart failure

Since last year, we have constructed and updated a database of approximately 4,800 patients who have been hospitalized for cardiac catheterization and treatment. This database has been analyzed with structural equation modeling or covariance structure analysis by adjusting analysis of moment structures, a statistical analysis method that has previously been known but has been rarely reported to be used in cardiovascular medicine. In particular, we have analyzed the relationships of clinical factors that cannot be expressed by multivariate statistical analysis alone, focusing on statistical analysis research on clinical data, including plasma B-type (brain) natriuretic peptide. With a path diagram the visual understanding of the relationships among factors is simplified and the multiple regression analysis and path analysis (repetition of multiple regression analysis) with factors and confirmatory factor analysis are made easier. In addition, Bayesian structure equation modeling successfully describes these results and is expected to be a next-generation statistical procedure for large clinical trials. Subsequently, we performed a detailed data analysis of chronic heart failure and published various analytic results, such as anemia and heart failure and the relationship of uric acid levels and cardiac function. We will promote clinical research widely based on the experience acquired from daily clinical practice. With regard to the mechanism of these findings, they are being clarified with basic research.

Imaging Research Group

1. Study group on imaging

With the increasing number of cases involving transcatheter aortic valve replacement,

also vital as preoperative examinations for evaluating the aortic valve are cardiac computed tomography and echocardiography. From this valuable case information, we are seeking research agendas for clinical studies. Through other imaging methods, such as cardiac magnetic resonance imaging and myocardial isotope tests, we are continuing to seek research agendas for clinical studies and to analyze cardiomyopathy and arrhythmias. We have also been investigating cardiac function in patients with lysosome diseases, especially Fabry's disease, in collaboration with the Department of Pediatrics.

Molecular Biology Research Group

In addition to the various effects of natriuretic peptides (NPs) on cardiovascular systems, increasing attention is being paid to the possibility that NPs induce adipose tissue browning and activate thermogenic program. We established a direct intracellular temperature measurement system using a fluorescent thermoprobe and investigated the thermogenic effects of A-type (atrial) natriuretic peptide (ANP) on brown adipocytes. The thermoprobe was successfully introduced into rat brown adipocytes, and the temperature-dependent change in the fluorescence intensity ratio was significantly higher in ANP-treated adipocytes than in untreated controls. The ANP treatment increased levels of uncoupling protein 1 in a p38 mitogen-activated protein kinase-dependent manner. Intriguingly, these thermogenic actions of ANP were more prominent when brown adipocytes were incubated at 35°C than at 37°C. These findings reveal a previously underappreciated role for NPs in the compensatory thermogenic action when the core body temperature decreases owing to unfavorable hemodynamic conditions in a state of severe heart failure. We are now investigating whether ANP exerts significant effects on adipose tissues in vivo.

Although fatty acids are involved in the predominant metabolic pathway of the healthy adult heart, glucose becomes an important preferential substrate for metabolism under specific pathological conditions, such as ischemia-reperfusion injury. We have previously reported that sodium glucose co-transporter (SGLT) 1 is expressed at high levels in both human and murine hearts and have found in a murine Langendorff model that cardiac SGLT1 significantly contributes to cardioprotection against ischemia-reperfusion injury. We are now investing the regulation and functional significance of cardiac SGLTs under insulin-resistant conditions.

Cardiac Physiology Research Group

By examining human hearts obtained at autopsy, we have found, with a immunohistological method, the presence of thrombin, the final product of the coagulation cascade. Coagulability is increased in patients with dilated cardiomyopathy (DCM). In knock-in mice with a cardiac troponin T deletion mutation that causes human DCM (ΔK210 knock-in mouse (B6; 129-Tnnt2^{tm2Mmto})), we assessed the effects of a direct thrombin inhibitor, dabigatran. Dabigatran significantly improved fractional shortening in echocardiographic findings and survival outcomes. From these results, we conclude that tissue thrombin is involved in the pathogenesis of DCM and that thrombin inhibition can be beneficial for the treatment of DCM. To investigate the hemodynamics of thrombin, HiLyte^{TM}-thrombin (AnaSpec, Fremont, CA) was administered to mice so that whole in-vivo imaging could be performed. The HiLyte^{TM}-thrombin was internalized to hearts and livers. Because we

did not detect messenger RNA of prothrombin in heart tissue by means of real-time polymerase chain reaction, we believe that the tissue thrombin is derived not from the heart (namely internal prothrombin) but from blood.

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General Summary

The number of patients we see in our department per month in now 15,000 and is increasing every year. We mainly see patients with diabetes (including 10% of type 1 diabetes) or endocrinological disorders. Clinically, we attempt to provide best healthcare to our patients with research evidence, clinical expertise, and patients' preferences. To accomplish this goal, we encourage the members of our division to perform basic and clinical research of high quality. With respect to education, we accept international students from other institutions. We encourage our trainees to improve their presentation skills. Finally, we strongly encourage our investigators to write manuscripts.

Research Activities

Diabetic complications
1. Molecular mechanisms governing intracellular signal transduction focusing on cell types relevant to the complications of diabetes
2. Roles of small guanosine triphosphate (GTP)-binding protein Rho and Rho-kinase in renal, retinal, neuronal, and endothelial biology
3. Isoform-specific roles of Rho-kinase in pathogenesis of the complications of diabetes
Approaches to this study range from in vitro to in vivo using gene-targeting approaches in mice

Epidemiology
1. Clinical trials of the treatment of patients with diabetes using continuous and flash glucose monitoring
2. 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
3. A population-based study of childhood obesity, insulin resistance and diabetes in the elderly, and genetic factors has also continued in Niigata Prefecture
4. Epidemiological study using data from more than 6,000 patients with diabetes from the 4 hospitals affiliated with The Jikei University

Molecular biology for pancreatic islets

Type 2 diabetes is known as a "bi-hormonal disorder" because of the dysregulated insulin and glucagon secretion. Reduced β cell mass is a major cause of dysregulated insulin secretion. Although a combination of elevated levels of glucose and free fatty acids (glucolipotoxicity) strongly induces β cell dysfunction and cell death, the underlying cause remains unclear. Also unclear is the precise molecular mechanism of glucagon in α cells. We found that serine/threonine kinase protein kinase c (Pkc) δ is involved in β cell death and glucagon secretion from α cells. Ongoing projects are as follows.

1. Molecular mechanisms of Pkcδ-dependent β cell mass reduction under conditions of glucolipotoxicity using β-cell-specific Pkcδ knockout mice and the insulin-secreting mouse insulinoma (MIN6) cell line
2. Involvement of the Pkcδ-pancreatic duodenal homeobox 1 (Pdx1) pathway studied with the chemical and genetic inhibition of Pkcδ in the MIN6 cell line
3. Molecular mechanisms of Pkcδ-dependent glucagon secretion under high-glucose conditions in the glucagon-secreting αTC1 cell line
4. The association of Pkcδ and peptide tyrosine tyrosine signals in glucagon secretion using the αTC1 cell line
5. Pkcδ function of insulin resistance in α cells studied with the chemical and genetical inhibition of Pkcδ in αTC1 cell line and islets
6. To elucidate the molecular mechanism of Pkcδ *in vivo*, establishment of α-cell- specific Pkcδ knockout mice
7. Physiological and histological characterization of deficiency of the gene protein kinase C, delta (*Prkcd*), in α cells under the diabetic condition

Endocrinology

1. Basic research

1) The role of 12-lipoxygenase in diabetic cardiomyopathy
2) The role of baroreflex sensibility on diabetic macroangiopathy, especially the effects of glycemic variability and blood pressure variability
3) Effect of a sodium-dependent glucose co-transporter (SGLT) 2 inhibitor in a rat model of diabetes
4) Effect of aldosterone in macula lutea degeneration

2. Clinical research

1) Effect of SGLT-2 inhibitor in patients with diabetes
2) The role of baroreflex sensibility in patients with diabetes
3) The durability of basal insulin affects day-to-day glycemic variability assessed by continuous glucose monitoring in patients with type 2 diabetes
4) Investigation of HbA1c, blood pressure, and body weight variability in patients with type 2 diabetes
5) Achievement of HbA1c and blood pressure and low-density lipoprotein-cholesterol goal of patients with type 2 diabetes (the Japan Diabetes Clinical Data Management Study Group)

Publications

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General Summary

The immediate goals of our clinical and basic research are to investigate basic and clinical aspects of malignant diseases and to try to improve outcomes for patients with solid tumors and hematological malignancies, leading to the ultimate goals of improving the natural history of malignant diseases. We have also been performing several clinical trials and basic research studies successfully throughout 2018.

Research Activities

Leukemias
Many patients with previously untreated hematological disorders have been referred to our department. The patients treated in 2018 included 22 patients with acute myeloid leukemia or acute lymphoblastic leukemia and 14 patients with chronic myeloid leukemia. We have performed clinical trials as a member of the Japan Adult Leukemia Study Group, which is a distinguished group established more than 20 years ago in Japan for clinical research and the treatment of such disorders.

Lymphomas
In 2018 we registered 94 patients with newly diagnosed cases of non-Hodgkin's lymphoma. We have performed clinical trials as a member of the Lymphoma Study Group of the Japan Clinical Oncology Group (JCOG). The study JCOG0601 (newly diagnosed low-risk advanced diffuse large B-cell lymphoma: phase II/III) was a pivotal protocol study beginning in 2007.

Myeloma
We registered 14 patients with newly diagnosed multiple myeloma in 2018. Numerous agents, which range from immunomodulatory drugs and proteasome inhibitors to monoclonal antibodies, have now been integrated into both induction and salvage regimens and have dramatically revolutionized the treatment landscape of multiple myeloma.
In-house protocols are also under investigation. A phase II study of a regimen of cyclophosphamide, bortezomib, and dexamethasone (CVD) is in progress for patients with newly diagnosed multiple myeloma.

Hematopoietic stem cell transplantation

To investigate and establish safer and more effective methods of hematopoietic stem cell transplantation, we have performed serial clinical studies examining umbilical cord blood transplantation, reduced-intensity stem cell transplantation from haploidentical donor, and an investigation of the mechanisms of graft-versus-host disease in hematopoietic stem cell transplantation.

Solid tumors

Many patients with solid cancers have been referred to our department from related divisions or departments from both inside and outside our hospital. Several of our studies seeking improved therapeutic outcomes are in progress throughout our university hospital with related divisions or departments. Since 2008 we have been investigating a combined chemotherapy with docetaxel, cisplatin, and 24 hours' continuous infusion of fluorouracil (5-FU) (the DCF regimen) for patients with advanced esophageal cancer. The study has been completed, and its results have recently been published. An improved protocol was launched 4 years ago and is now being investigated. A novel drug-development study of an orally decaying formulation of S-1 for patients with advanced gastric cancer co-operating was performed by us, in cooperation with a colleague department; the study has been completed, and the new formulation of S-1 has become available for standard clinical practice. Our first-line chemotherapy regimens for patients with advanced colorectal cancer are folinic acid, 5-FU, and oxaliplatin (FOLFOX) and folinic acid, 5-FU, and irinotecan (FOLFIRI). Antibodies became available against vascular endothelial growth factor (VEGF) in 2007 and against epidermal growth factor receptor (EGFR) in 2008, and combination therapies of these antibodies and FOLFOX or FOLFILI have also been performed. Because oral drugs are more convenient and safer, 5-FU is replaced by S-1 or capecitabine in such intravenous combination chemotherapy regimens as FOLFOX or FOLFILI, leading to the development of such improved regimens as S-1 and oxaliplatin (SOX), capecitabine and oxaliplatin (XELOX), irinotecan and S-1 (IRIS), and capecitabine and 3-weekly irinotecan (XELIRI). Salvage therapies using regorafenib or a combination of trifluridine and tipiracil (TAS-102) became standard care for resistant and refractory advanced colorectal cancer.

Basic research

One of our important activities is translational research on solid cancers and hematological malignancies. Because the clinical requirement is urgent, persistent research is warranted. Cancer fatigue is now an emerging issue for patients with advanced malignant disease. We have been evaluating, in collaboration with the Department of Virology, the correlation between cancer fatigue and human herpesvirus 6 reactivation in patient's salivary juice and blood samples. The preliminary results were reported at the annual meeting of the Multinational Association of Supportive Care in Cancer held in Miami, Florida, USA.

Life-threatening complications after treatment of disease seem to be one of major problems. Supportive care for patients with malignant disease is also extremely important. We

have focused on supportive care for years. Measuring renal tubular proteins in urine can predict renal damage caused by cisplatin. Therefore, we have been examining whether renal tubular proteins should be measured for the early detection of renal damage in patients undergoing cisplatin combination chemotherapy, such as docetaxel, cisplatin, and 5-FU (DCF) and gemcitabine, dexamethasone, and cisplatin (GDP). The studies are in progress.

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Department of Internal Medicine
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General Summary

We perform clinical and basic research concerning chronic obstructive pulmonary disease (COPD), bronchial asthma, pulmonary infection, pulmonary fibrosis, and lung cancer, which are closely associated with aging. We investigate the pathophysiology of lung diseases associated with aging, especially COPD and idiopathic pulmonary fibrosis (IPF), and the pathogenesis concerning cellular senescence and autophagy. We also collaborate with National Cancer Research Center concerning the detection of mutations of the epidermal growth factor receptor gene (*EGFR*), extracellular vesicles, and immune checkpoint inhibitors in the field of lung cancer treatment.

Research Activities

COPD

The condition known as COPD is caused by the noxious effects of tobacco smoke, which leads to airway epithelial cell injury and the induction of phenotypic changes Such changes as squamous metaplasia and cellular senescence are assumed to be part of the adaptive response to toxic components. Autophagy is a bulk degradation pathway for cellular components which is essential for the maintenance of cellular homeostasis. When autophagy is impaired, damaged proteins and organelles accumulate and lead to cell death and cellular senescence. Cell death and cellular senescence are believed to be involved in the pathogenesis of COPD.

Mitochondria are dynamic organelles that are essential for cellular metabolic functions and continuously change their shape through fission and fusion. The proper regulation of mitochondrial dynamics is crucial for the maintenance of functional mitochondria and, hence, the disruption of dynamics induces excessive reactive oxygen species production, resulting in apoptosis, necroptosis, and cellular senescence. We reported that expression of PINK1/PARKIN, which mediate mitophagy, were decreased in lung epithelial cells from patients with COPD, and that PARKIN knockout mice were susceptible to the development of emphysema in a mouse model of smoking‐induced COPD. We also reported that lamin B1 expression of bronchial epithelial cells was decreased in specimens from patients with COPD and was decreased and resulted in cellular senescence in a mouse model of smoking‐induced COPD.

Bronchial Asthma

Because most evidence regarding postoperative pulmonary complications had been established more than 10 years ago, we have recently evaluated perioperative management using new inhalant drugs in patients with COPD and asthma. We reviewed physiological backgrounds, surgical factors, and perioperative-specific treatment for COPD and asthma. Risk factors for postoperative pulmonary complications are a history of smoking and severe asthma in patients with asthma and are age, upper abdominal surgery, and long operation time in patients with COPD. Therefore, these complications could be reduced by adequate treatment with inhaled corticosteroids in patients with eosinophilic asthma and by introducing treatment for COPD in patients with COPD.

IPF

We have produced evidence that lungs with IPF show enhanced epithelial cell senescence, including aberrantly re-epithelialized bronchial cells. Playing an important regulatory role in cellular senescence and differentiation is autophagy. We have also found that insufficient autophagy is a potent underlying pathology of both accelerated cellular senescence and myofibroblast differentiation in IPF. Insufficient mitophagy leads to the accumulation of injured mitochondria, which produce excessive reactive oxygen species.

Alveolar epithelial cell (AEC) injury leading to cell death is involved in the process of fibrosis. The excessive apoptosis of AECs has been widely implicated in the pathogenesis of IPF. Necroptosis is a type of regulated/programmed necrosis. A key regulatory role in initiating necroptosis is played by a multiprotein complex composed of receptor-interacting protein kinases (RIPKs) 1 and 3. We found that expression levels of RIPK3 were increased in lungs with IPF and that both apoptosis and necroptosis were detected mainly in AECs. Treatment with bleomycin induced RIPK3 expression in AECs and increased levels of high mobility group box 1 and interleukin 1β in the lungs of mice. Bleomycin-induced lung inflammation and fibrosis were attenuated in RIPK3 knockout mice with a concomitant reduction in high mobility group box 1 and interleukin 1β. Therefore, we concluded that RIPK3-regulated necroptosis in AECs is involved in the mechanism of lung fibrosis development through the release of damage-associated molecular patterns as the pathogenesis.

We also reported interstitial pneumonia associated with psoriasis, which is a chronic and recurrent inflammatory skin disease. Inflammatory processes might extend outside the skin and affect the lungs. We clearly demonstrated the relationship of psoriasis and interstitial pneumonia.

Lung cancer

Noninvasive monitoring of EGFR mutations conferring sensitivity and resistance to tyrosine kinase inhibitors (TKIs) is vital for the efficient treatment of lung adenocarcinoma. Although plasma circulating cell-free tumor DNA can be detected at an early stage, the size of the tumor does not strongly correlate with the concentration of whole cell-free DNA, including normal leucocyte DNA. The analysis of cell-free DNA from patients with acquired TKI-resistance disease or extrathoracic disease has shown that progression is correlated with a high detection rate of TKI-sensitive mutations. We concluded that

cell-free DNA in patients with EGFR-TKI-resistance or extrathoracic disease progression might be useful for the analysis of cancer genomics.

Biomarkers are urgently required for predicting the effects against non-small-cell lung cancer of monoclonal antibodies acting as immune-checkpoint inhibitors against programmed cell death (PD) 1 and PD-L (PD ligand) 1. We investigated the significance of the plasma level of soluble (s) PD-L1 being used as a biomarker for the therapeutic effects of nivolumab, an anti-PD-1 monoclonal antibody. The baseline plasma sPD-L1 concentration was determined with an enzyme-linked immunosorbent assay. A complete or partial response was achieved by 59% of 39 patients with low plasma sPD-L1 levels and by 25% of those with high plasma sPD-L1 levels. In addition, progressive disease developed in 22% of patients with low plasma sPD-L1 levels and 75% of those with high plasma sPD-L1 levels. The time to failure and overall survival were significantly longer for patients with low plasma sPD-L1 levels than for patients with high plasma sPD-L1 levels. The clinical benefit from nivolumab therapy was significantly associated with baseline plasma sPD-L1 levels. Plasma sPD-L1 levels might represent a novel biomarker for predicting the efficacy of nivolumab therapy against non-small-cell lung cancer.

Extracellular vesicles in lung diseases

Extracellular vesicles (EVs), such as exosomes, play an important role in intercellular communication. Recently, the involvement of EVs in the pathogenesis of lung diseases have been examined. The EVs include numerous DNA, proteins, messenger RNAs, and microRNAs that can regulate intercellular communication. Various kinds of respiratory cells release EVs that can have protective or detrimental functions, depending on the type of donor cells, type of stimuli, and components. In lung cancer, tumor-derived EVs carry multiple immunoinhibitory signals, disable antitumor immune effector cells, and promote tumor escape from immune control. The EVs can also maintain airway homeostasis, induce proinflammatory effects, and promote antigen presentation, thereby regulating lung inflammation and immune responses. Therefore, EVs play important roles in the pathophysiology of inflammatory lung diseases. We have addressed the role of EVs in the pathogenesis of asthma, COPD, and IPF and in the treatment of lung cancer.

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Department of Internal Medicine
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General Summary

1. Management of a database of our medical examinations and treatments
2. Planning a postgraduate training program to acquire skills for the general practice required in the community

Research Activities

Division of General Medicine, The Jikei University Hospital
We have been constructing a database of our outpatient clinic including information concerning reason for visiting, symptoms, initial diagnoses, and initial treatments. Frequent reasons for consultation are abdominal pain, cough, and pyrexia. Frequent initial diagnoses are upper respiratory tract infection, infectious gastroenteritis, and headache. These data are expected to be useful for analyzing trends in primary care at large general hospitals.
Recently, medical education has expanded to first-line health care including community health care, in addition to hospital-based specialized medicine. Thus, we are planning the postgraduate and continuing professional development of physicians to acquire skills for the general practice required in the community.

Division of General Medicine, The Jikei University Katsushika Medical Center
We have treated 2 cases: a case of acquired immunodeficiency syndrome presenting pancytopenia after the onset of pulmonary abscess, and a case of type 2 diabetes and refractory eczema which improved after human immunodeficiency virus infection had been treated.
We also continue basic research on gas biomarkers from pulmonary expiration in patients with inflammatory disease and collagen disease. We found the specific pattern of materials from expiration gas.

Division of General Medicine, The Jikei University Daisan Hospital
We found that presepsin levels are high in patients with sepsis and pseudogout.
We discussed the “do not attempt resuscitation” order and the physician order for life sustaining treatment (POLST) in university hospitals and community hospitals. Almost all physicians and nurses know the “do not attempt resuscitation” order, 60% of physicians and nurses were confused in actual cases. Only 2% of them knew the POLST.

Division of General Medicine, The Jikei University Kashiwa Hospital

We have classified fibromyalgia into several clinical categories and investigated the suitable treatment for each category.

We are performing research concerning the Japanese version of the POLST in Japanese hospitals and advanced care planning in rural areas. Data sampling finished within 2018.

Concerning the management of the Hospital Ethics Committee and Clinical Ethics Consultation in The Jikei University Kashiwa Hospital, there were 10 consultations last year, and these cases were under reconsideration for presentation.

We conducted open seminars related to clinical ethics 4 times in the past year.

Publications

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Department of Psychiatry

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General Summary

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

Research Activities

Psychogeriatric group
We are performing several research studies investigating the neural basis of neuropsychiatric symptoms and social functions in patients with neurodegenerative diseases and elderly patients with psychiatric disorders. Methods of examination include neuropsychological testing; neuroimaging, such as brain magnetic resonance imaging and single-photon emission computed tomography; and genetic testing. We continue to study changes in the DNA methylation level as a biomarker of neurodegenerative diseases; we now focus on the effects of DNA methylation on the appearance of behavioral and psychological symptoms. We are also continuing multicenter collaborative research to develop methods for the early diagnosis of frontotemporal lobar degeneration and to assess clinical and genetic factors affecting its natural history. Also, in collaboration with the National Institute of Radiological Sciences, we are conducting research on tau imaging of neurodegenerative diseases and psychiatric symptoms. We are also studying the validity of treatment for patients with type 2 diabetes and cognitive decline.

Morita therapy group
In cooperation with psychotherapists of other methods such as cognitive-behavioral therapy and psychoanalysis, we have been developing programs and materials to train young psychiatrists to master the basic techniques of the clinical interview. We continued the following studies this year: (1) practical research towards obsessive compulsive disorder with autistic spectrum disorder, (2) practical research towards the application of Morita therapy to adolescent patients and patients with *hikikomori* (withdrawal), (3) the psychopathology of social anxiety disorders, (4) factors in the recovery of patients with depression through inpatient Morita therapy, (5) the application of Morita therapy to elderly patients, and (6) the application of Morita therapy for palliative care.

Psychopharmacology group
In basic research, we performed the following studies in rodents: (1) the formation mechanism of drug addiction, (2) the neural basis of addiction-related impulsivity, and (3) the development of a novel anticraving agent. These studies were performed in collaboration with the NTT Communication Science Laboratories and the Department of Psychology, Senshu University. In clinical research, we performed the following studies in humans: (1) biological, psychological, and social predictors of recovery in patients with schizophrenia; (2) regulation of the salience network by antipsychotic agents through dopamine D2/3 receptors with resting state functional magnetic resonance imaging; and (3) qualitative research on adherence in patients with schizophrenia. Integration between basic and clinical research is a fundamental concept of the Psychopharmacology group.

Clinical electroencephalography group
We attempted to interpret, on the basis of Neojacksonism (proposed by Henri Ey), cases in which psychotic symptoms associated with epilepsy were presented. Furthermore, we reported changes in serum concentrations of antiepileptic drugs (especially new ones) during the pregnancy of patients with epilepsy. A study was performed to prevent depression from recurring in patients with epilepsy. We examined the safety and efficacy of psychotropic drugs in several forms of psychosis associated with epilepsy.

Psychophysiology group
Studies examined: (1) changes in sleep structures due to cognitive behavioral therapy for insomnia using the cyclic alternating pattern method, (2) the efficacy of group cognitive behavioral therapy for primary insomnia and depression, (3) the effects of Chinese herbal medicine on sleep disorders, and (4) the biomarkers of fatigue in obstructive sleep apnea syndrome.

Neuromodulation group
Approximately 1 million patients with mood disorders are receiving medical treatment in Japan. However, few treatments are available for patients with treatment-resistant depression or bipolar depression. Our mission is to relieve symptoms in these patients by means of a noninvasive neuromodulation technique, such as repetitive transcranial magnetic stimulation (rTMS). Additionally, we seek to develop a medical device for filling unmet needs in cooperation with domestic and overseas companies and to promote regulatory science research.
The tasks that we are currently involved in are as follows: (1) postmarketing surveillance study of the efficacy and safety of rTMS devices in Japan, (2) expanding indications for bipolar depression using the Japanese advanced medical system, (3) research and development of maintenance therapy using rTMS, (4) research and development of a new measurement to treat depression, (5) research on the efficacy of rTMS in combination with a return to work program, (6) research and development of magnetic seizure therapy, and (7) research and development of computerized cognitive training.
We established a neuromodulation laboratory in September 2017 and are now preparing these research studies and clinical practice for the next fiscal year.

General hospital psychiatry group

In a study of interventional treatment based on cognitive-behavioral therapy aimed at preventing recurrences of depression, a computer system and sleep evaluation methods were introduced in addition to a previous evaluation system for more effective presentations and for more precise estimation. Also investigated were new indications for this intervention for atypical depression, bipolar depression, and insomnia. Another study investigated the issues associated with mental care services for patients with cancer of the digestive tract.

Psychopathology, psychotherapy, and child study group

We have twice provided supervision (supervisor: Dr. Sadanobu Ushijima; case presentations: Drs. Masanori Kawakami and Hikaru Seto) to residents to cultivate a psychotherapy mindset. We gave several case presentations at the 41st annual meeting of the Japanese Society of Psychopathology and at the 11th annual meeting of the Japanese Society of Anxiety and Related Disorders. Our strategy moving forward will be to (1) continue supervision, (2) examine the usefulness of the "desire for life" (Masatake Morita) in the postmodern era, and (3) research subtypes of Morita's neurosis in the postmodern era.

Clinical psychology group

We have continued to discuss and study psychotherapeutic processes and the techniques of cognitive behavior therapy, art therapy, therapeutic assessment, Morita therapy, relief care, psycho-oncology, and social skill training. We have also examined the characteristics of developmental disorders and higher brain dysfunctions through psychological assessments. Furthermore, we have trained graduate students in a clinical psychological course.

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General Summary

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

Research Activities

Inherited metabolic disease group
We continue to study gene therapy projects for mucopolysaccharidosis type II. In a project funded by the Japan Agency for Medical Research and Development, the optimal lentivirus vector was selected for hematopoietic stem cell-targeted gene therapy. We started to generate a lentiviral vector for a nonclinical study and to optimize transduction of human hematopoietic stem cells. In another agency-funded gene therapy project for mucopolysaccharidosis type II, significant reductions of accumulated compounds in the brain were observed by intravenous administration of adeno-associated virus vectors in a murine model. In addition, we are developing gene therapy for GM1 gangliosidosis and have started a study of Fabry's disease using artificial intelligence.

Neurology group
We are conducting research on Dravet syndrome and epilepsy related to the protocadherin 19 gene (*PCDH19*) by using disease-specific induced pluripotent stem cells and knockout rats. The aims of our research include clarifying the molecular and cellular pathology and exploring a therapeutic availability of cell transplantation. By using manganese-enhanced magnetic resonance imaging, we have successfully identified a novel finding in the Dravet syndrome rat brain, which might be related to the epileptogenesis of Dravet syndrome. In clinical research, we have found that high frequency oscillation in electroencephalograms of the scalp can be an early sign of atypical evolution of childhood epilepsy with centrotemporal spikes; we have also characterized a developmental alteration of regional cerebral blood flow in childhood.

Nephrology group
The main subjects of our research are as follows: (1) to estimate human total nephron number using a combination of image analysis and renal biopsy; (2) to investigate the independent risk factors for acute kidney injury after hematopoietic stem cell transplantation; (3) to investigated whether a combination of ACK2, an anti-c-kit antibody, with a low-dose irradiation conditioning regimen is effective for hematopoietic stem cell-targeted gene therapy for mucopolysaccharidosis type II mice; and (4) functional analysis of mutations of laminin subunit beta 2 (*LAMB2*) in children with asymptomatic proteinuria.

Neonatology group
We have developed a new respiratory support device for neonates on the basis of the fluid dynamics theory. We are now assessing the function of this device for its clinical usefulness. The other basic study is time-solved near-infrared spectroscopic measurement of tissue oxygen. This technology is a new way for monitoring oxygen and hemodynamics in live brain tissue. We have studied lectin-like oxidized low-density lipoprotein receptor 1, which is recognized as a biomarker of the severity of hypoxic-ischemic encephalopathy, with the National Center of Neurology and Psychiatry. We are preparing for an international study of the effect of music therapy for neonates with cerebral palsy.

Infectious diseases and Immunologic Disorders group
We investigated the anti-inflammatory treatment for colitis associated with chronic granulomatous disease. Furthermore, we examined the sensitivity and specificity of pathogenic genome sequence analysis in patients with severe infection. Although a culture test of blood is a useful and reliable method of identifying pathogenic bacteria and fungi in patients with sepsis, it has several problems, such as detection sensitivity, a long duration for culture, and the development of an appropriate culture medium.

Hematology and Oncology group
We have prepared to perform a phase II international clinical study for patients who are children, adolescents, or young adults in whom acute promyelocytic leukemia was newly diagnosed. We are starting phase I and II clinical studies of dendritic cell therapy for therapy-resistant pediatric brain tumors. We demonstrated the molecular mechanism of development of esophageal squamous cell carcinoma in the patient with chronic graft-versus-host disease and prolonged administration oimmunosuppressants. Moreover, we demonstrated that therapy targeting the nuclear factor kappa B signaling pathway is effective for enhancing or restoring the sensitivity to bromodomain inhibitor I-BET151 in U937 cells.

Cardiology group
The research studies have included the following: (1) evaluation of the mechanism of reverse remodeling in the status of heart failure during the growth period, (2) calculation of the shunt flow in a rat model of aortopulmonary collateral artery with left pulmonary artery ligation under hypoxia environment, (3) the 2nd group with pulmonary hypertension of a rat model of left atrium stenosis, (4) utility of urine titin to detect pediatric myo-

cardial damage, (5) evaluation of right ventricular fibrosis using 2-dimensional speckle tracking and diffusion tensor imaging in mice with right ventricular pressure overload, (6) the role of hypoxia-inducible factor 1α in the pulmonary artery smooth muscle of mice with hyperoxia-induced neonatal lung injury, (7) the drug stress test utility of long QT syndrome and (8) validation of a pediatric index of a mortality score of 3 in the pediatric intensive care unit.

Allergy group

The main subjects of our research are as follows: (1) the role of eosinophil, mast cells, and epithelial cells in the pathology of allergic diseases; (2) pediatric asthma; (3) food allergy; (4) atopic dermatitis; (5) treatments for allergic diseases; and (6) prevention of allergic diseases. We are performing some multicenter randomized controlled trials: the Daily versus intermittent Inhaled Fluticasone in Toddlers with recurrent wheezing (DIFTO) study and the Efficacy of a moisturizing cream in the treatment of atopic dermatitis in children (MADEC) study.

Endocrinology group

We conducted the first alanine scanning mutagenesis study, in which 132 alanine variants located in the paired domain of the thyroid-specific transcription factor paired box family protein 8 were created and systematically evaluated *in vitro*. We found that 76 alanine variants (55%) were loss-of-function variants, which were of skewed distribution and were more frequently observed in the N-subdomain than in the C-subdomain. We performed a survey of the present condition of fetal goitrous hypothyroidism in Japan. We found 31 patients in whom fetal goitrous hypothyroidism had been treated with intrauterine injection of levothyroxine in the past 20 years.

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Department of Dermatology

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General Summary

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

Research Activities

Psoriasis
Various systemic therapies, including oral cyclosporin microemulsion preconcentrate, methotrexate, etretinate, biologics, and topical therapies, such as vitamin D3, and corticosteroids, have been used, depending on disease severity and the degree to which quality of life (QOL) is impaired in individual patients. Also, phototherapy is effective and has been performed in our skin-care clinic. We have evaluated patients' QOL and have developed Japanese versions of the Psoriasis Disability Index and the Work Productivity and Activity Impairment questionnaire for psoriasis. In a special psoriasis clinic, we select patient-based treatments to satisfy patients' demands. Biologic agents, including infliximab, adalimumab, ustekinumab, secukinumab, brodalumab, ixekizumab, and guselkumab, are available and have been used to treat severe, intractable psoriasis. Clinical trials have been performed with new biologic agents and new topical agents.

Atopic dermatitis
Recently, psychosocial factors have been suggested to affect the exacerbation of atopic dermatitis. Therefore, we are treating patients on the basis of both evidence-based medicine and QOL issues. We obtain a precise medical history from each patient and evaluate the degree of QOL impairment. We are also doing some basic experiments with a mouse model of atopic dermatitis to reveal the mechanism of pruritus in this disease. An antibody agent (dupilumab) against the interleukin (IL)-4/IL-13 receptor is available and has been used to treat moderate-to-severe atopic dermatitis. Clinical trials have also been performed of a topical phosphodiesterase 4 inhibitor and an antibody agent against the IL-31 receptor.

Malignant skin tumors
We have been studying clinical courses, postoperative outcomes, and genomic and

expression changes in patients with malignant melanoma, extramammary Paget's disease, squamous cell carcinoma, basal cell carcinoma, cutaneous T-cell lymphomas, and a wide variety of soft-tissue sarcomas, including malignant peripheral nerve sheath tumors. For the accurate diagnosis of pigmented tumors, we always perform dermoscopic examinations and sentinel lymph-node biopsies. For patients with advanced disease, we have performed multidisciplinary treatment, including immune check point inhibitors, molecular targeted agents, chemotherapy, and radiation therapy.

Neurofibromatosis
Because the number of registered patients in our clinic is the largest in Japan, we concentrate on long-term follow-up and the improvement of impaired QOL by means of accurate diagnosis and the resection of neurofibromas. The estimated lifetime risk of malignant peripheral nerve sheath tumor (MPNST) in patients with neurofibromatosis 1 is approximately 10%. We have used the methylation-specific polymerase chain reaction (PCR) and real-time reverse transcriptase PCR to analyze the methylation status of tumor suppressor genes and cancer-testis genes in established MPNST cell lines.

Herpes virus infection
1. Herpes simplex virus
Rapid diagnostic procedures by means of immunohistochemical staining with monoclonal antibodies against herpes simplex virus 1 and 2 and varicella-zoster virus are performed in this clinic. After the diagnosis is confirmed, suppressive therapies with valaciclovir are started to improve QOL.
2. Herpes zoster and postherpetic neuralgia
Initial treatments are performed in this clinic for herpes zoster and postherpetic neuralgia (PHN). To prevent PHN, we prescribe tricyclic antidepressants. Posthoc analyses of a subgroup of patients have shown that amitriptyline in combination with acyclovir reduce the incidence of PHN. We prescribe pregabalin, tricyclic antidepressants, selective serotonin reuptake inhibitors, and opioid analgesics, such as Tramacet® (Grünethal Ltd., Stokenchurch, UK), which contains tramadol hydrochloride and acetaminophen.

Human papillomavirus infection
In addition to ordinary cryotherapy, agents that have been used to treat viral warts include topical vitamin D3, salicylic acid, glutaraldehyde, and monochloroacetic acid. Contact immunotherapy with squaric acid dibutylester, CO_2 lasers, and pulsed dye lasers has also been used to treat severe intractable viral warts. Human papillomavirus infection typing with the PCR has regularly been performed.

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

Laser
The Q-switched 694-nm ruby laser is useful for treating nevus of Ota, acquired dermal

melanocytosis, and ectopic Mongolian spots. On the other hand, nevus spilus/café-au-lait spots are difficult to treat with this laser because they often recur after 1 to 2 months. The recently introduced 595-nm V-beam laser (long pulsed dye laser) is effective for treating intractable vascular lesions. The ultrapulse CO_2 laser can be used to quickly remove lesions of actinic keratosis, seborrheic keratosis, syringoma, and epidermal nevus.

Skin Care Clinic

Narrow-band ultraviolet B irradiation is performed for patients with psoriasis, alopecia, atopic dermatitis, prurigo nodularis, vitiligo, or cutaneous T-cell lymphomas. Other special clinics, including those for skin care lessons, therapeutic make-up, acne care, mental care, and *kampo* medicine, are available for patients on demand.

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Research Activities

Division of diagnostic imaging

1. Differentiating between glioblastomas with or without mutation of the isocitrate dehydrogenase gene from imaging findings

Along with the revision of the 2016 World Health Organization classification of brain tumors, molecular genetic variables began to be used. In particular, mutation of the isocitrate dehydrogenase gene (*IDH*) is an important element in glioma classification. We examined whether the imaging findings can differentiate between glioblastomas with or without *IDH* gene mutation.

2. Imaging features of cystic neck lesions: cystic lymph node metastasis from human papillomavirus–positive oropharyngeal cancer, the 2nd branchial cyst and tuberculous lymphadenitis

Human papillomavirus and oropharyngeal cancer are frequently associated with cystic lymph node metastasis. We evaluated the differences of imaging features among cystic lymph node metastasis from human papillomavirus and oropharyngeal cancer and 2 non-malignant cystic neck lesions: the 2nd branchial cyst and tuberculous lymphadenitis.

3. Determining the clinical and characteristic computed tomographic findings of airspace enlargement with fibrosis

A total of 800 patients with chronic obstructive pulmonary disease were evaluated retrospectively with inspiratory and expiratory computed tomographic (CT) scans. Nine patients had multiple cysts that were significantly decreased in size and were probably corresponding to airspace enlargement with fibrosis.

4. Evaluation of the insufficient filling of the left atrial appendage on cardiac CT in patients with atrial fibrillation.

We compared CT images of patients with or without defects to clarify possible predictors of left atrial appendage filling defects. Persistent atrial fibrillation and left atrial appendage volume on cardiac CT images are independent predictors.

5. Ovarian seromucinous borderline tumors: usual and unusual magnetic resonance imaging findings

Magnetic resonance imaging (MRI) findings of 26 patients with 32 ovarian seromucinous borderline tumors were reviewed. Such tumors are usually a cystic tumor with a solid component, which shows high signal intensity (SI) on T_2-weighted images with a high apparent diffusion coefficient (ADC) value. However, in some cases the tumors were purely cystic or solid.

6. Breast MRI for prediction of lymphovascular invasion in patients with breast cancer and clinically negative axillary lymph nodes
The tumor ADC value, the peritumoral ADC value, and the peritumor-tumor ADC ratio were predictive findings for lymphovascular invasion in patients 55 years or younger.
7. Study of quantification of rheumatoid arthritis with dual-energy CT
We will examine the usefulness of dual-energy CT quantitative evaluation in the activity of rheumatoid arthritis compared with semiquantitative evaluation with contrast-enhanced MRI.
8. The anatomical evaluation of the findings of dual-energy CT of psoriatic arthritis using normal cadaver finger
We compared the dual-energy CT iodine mapping findings of psoriatic arthritis with high-resolution MRI imaging and the macroscopic appearance of a normal finger from a cadaver.

Division of Nuclear Medicine
Diagnostic value of ultrasonography and Tl-201/Tc-99m dual scintigraphy in differentiating between benign and malignant thyroid nodules.
To evaluate the performance of ultrasonography and Tl-201/Tc-99m dual scintigraphy in differentiating benign and malignant thyroid nodules. An ill-defined margin and microcalcification on ultrasonography were independent predictors of a malignant thyroid nodule. A persistent pattern seen on Tl-201/Tc-99m dual scintigraphy can contribute to the differential diagnosis.

Division of Interventional Radiology
Feasibility of 4-dimensional digital subtraction angiography for renal arteriovenous malformation
We retrospectively evaluated the usefulness of 4-dimensional subtraction angiography for renal arteriovenous malformation compared with that of 3-dimensional digital subtraction angiography as an initial experience.

Division of Radiation Therapy
1. To clarify the optimum fractionated radiotherapy of cancer with nonuniform radiosensitivity using the general linear quadratic model
The number of tumor cells before radiotherapy is important for local control after radiotherapy. Therefore, the number of tumor cells per unit volume was measured in cases of breast cancer for which surgery was performed. Given nonuniform radiosensitivity to the model tumors, estimate local control rate with the general linear quadratic model. https://radbiolog.jp
2. Dose finding and confirmatory trial of superselective intra-arterial infusion of cisplatin and concomitant radiotherapy for patients with locally advanced maxillary sinus cancer
We have started research for a new concept and are evaluating local control for more advanced local tumors, the normal-tissue conservation rate, and late complications.
3. The safety and effectiveness of a new enzyme-targeting radiosensitization treatment for bulky cervical cancers

A novel enzyme-targeting radiosensitizer for hypoxic tumors comprised of hydrogen peroxide and sodium hyaluronate has been developed by Yasuhiro Ogawa, Hyogo Prefectural Kakogawa Medical Center. The purpose of this study was to evaluate the safe achievement of bioradiotherapy and the effectiveness of Kochi Oxydol-Radiation Therapy for Unresectable Carcinomas, Type II (KORTUC II).

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Department of Surgery
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General Summary

The delivery of research papers is supported by writing skills in addition to the ability to accomplish the study. More efforts to read scientific papers are necessary to improve writing skills and to ensure patient safety. All surgeons should keep in mind that research based on anatomic, pathologic, and physiologic principles, in combination with animal experimentation, makes it possible to develop complex operative procedures and to become the consummate surgeon, as stated in the last Southern Surgical Association Presidential Address (J Am Coll Surg 2015; 220(4); 387‑395).

Research Activities

Upper gastrointestinal surgery

We evaluated the effects of preoperative chemotherapy with fluorouracil, cisplatin, and docetaxel for resectable advanced esophageal cancer on postoperative outcomes and long‑term survival. We continue to study how to reduce complications after esophagectomy. An intraoperative thermal imaging system and recurrent nerve integrity monitoring are used to assess the viability of the gastric tube to prevent anastomosis‑related complications and postoperative recurrent nerve palsy, retrospectively. Clarifying the usefulness of a near‑infrared fluorescence imaging system for the viability of the gastric tube is also planned. We had performed many laparoscopic operations for esophageal motor disorders and performed per‑oral endoscopic myotomy for achalasia. The preoperative and postoperative pathophysiology and the effects of these treatments were investigated with high‑

resolution manometry and multichannel intraluminal impedance pH monitoring. The significance of completely circumferencial myotomy for achalasia with severe chest pain is being evaluated.

We developed sentinel node navigation surgery using an infrared ray endoscopic system for the first time in the world. By means of this system, minimally invasive surgery with curability became possible.

Postgastrectomy syndrome comprises specific symptoms and is a target for treatment. To decrease the incidence and severity of postgastrectomy syndrome and to maximize residual gastric function, several types of limited gastric resection with refined techniques of reconstruction have been attempted. In addition, multiple tests, such as the Postgastrectomy Syndrome Assessment Study 37, are performed to evaluate the postgastrectomy gastrointestinal function of various gastrectomy procedures.

In basic research on esophageal cancer and gastric cancer, we have surveyed biological cancer behavior by means of DNA chips and immunohistochemical and reverse transcriptase-polymerase chain reaction methods.

Treatment for obesity is performed by a team (dietician, pharmacist, and physicians), and patients with resistant morbid obesity undergo surgery. The change of gastroesophageal reflux between presleeve and postsleeve gastrectomy is assessed with 24-hour pH monitoring.

Lower gastrointestinal surgery

In collaboration with the Department of Internal Medicine we hold conferences regularly and examine multimodal therapy for colorectal cancer. We have been investigating operative methods, complications, and histopathological factors with a database of patients with colorectal cancer. We started studies of anal function by means of stationary 3-dimensional manometry and aim at specific treatment for anal disease and postoperative complications. We are developing a complementary DNA library from surgical specimens and preparing a database for basic research.

Together with the Department of Biochemistry, we analyzed the expression of intracellular signal molecules that are associated with the progression and growth of cancer. We analyzed dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 (DYRK2) involved in the induction of apoptosis and the control of the cell cycle. By correlating with the database, we investigated the relationship of the expression of DYRK2 with associated genes. We prepared 3-dimensional cultures with colorectal cancer specimens to form so-called "organoid," with which we started basic research on the mechanism of drug action. Our aim is to develop methods to choose the appropriate medicines before treatment.

When chemoradiation therapy is given to patients with colon cancer, radiation causes microenvironmental inflammation around cancer cells and promotes the secretion of nuclear factor kappa B (NF-κB), which is associated with the growth and invasion of tumor cells and with angiogenesis. Recombinant thrombomodulin is known to have antitumor effects by inhibiting NF-κB for pancreas cancer cell lines in previous studies. We examined whether recombinant thrombomodulin has antitumor effects on colorectal cancer cell lines that are resistant to chemotherapy by inhibiting activation of NF-κB.

Hepatobiliary and pancreatic surgery

The outlines of our main research activities are as follows:

1. Living donor liver transplantation (LDLT) and regenerative medicine
2. Treatment for hepatocellular carcinoma (HCC) and control of recurrence
3. Chemotherapy for pancreatic and biliary cancers
4. Expansion of surgical indications for multiple hepatic tumors
5. Laparoscopic surgery for the liver, biliary tree, pancreas, and spleen
6. Navigation surgery for hepatobiliary and pancreatic diseases
7. Nutritional therapy for patients with cancer (enhanced recovery after surgery)
8. Control of surgical site infection
9. Effect of treatment with eltrombopag before splenectomy for idiopathic thrombocytopenic purpura
10. Molecular-targeting therapy for advanced HCC
11. Analyses of new biological tumor markers for HCC

Since 2007 we have performed LDLT for 22 patients, including 3 patients who underwent ABO-incompatible LDLT. All 22 recipients were discharged in good condition on postoperative days 15 to 146, the donors were discharged on postoperative days 7 to 26, and all donors have returned to preoperative status. We are planning to extend the indications of LDLT to acute hepatic failure. The outcome of patients who undergo HCC resection at our institution is much better than the national average. To reduce postoperative complications, we investigated the risk factors and effective treatments for postoperative portal vein thrombosis and venous thromboembolism. We have performed clinical trials for pancreatic cancer and biliary tract cancer. Ongoing trials for pancreatic cancer evaluate combination chemotherapy with gemcitabine, S-1 with regional arterial infusion of nafamostat mesilate for advanced pancreatic cancer, and gemcitabine in combination with regional arterial infusion of nafamostat mesilate as an adjuvant chemotherapy after pancreatectomy. Current trials for advanced biliary tract cancer include chemotherapy with S-1 every other day in combination with gemcitabine/cisplatin. We have also performed extended liver resection as a conversion therapy for multiple metastatic tumors of the liver, mainly originating from colorectal cancers. Furthermore, laparoscopic surgery, including hand-assisted laparoscopic surgery and laparoscopy-assisted surgery, i.e., hybrid surgery, has gradually been expanded for hepatobiliary, pancreatic, and splenic diseases because of its lower invasiveness. We have used the SYNAPSE VINCENT medical imaging system (Fujifilm Medical Systems) for 3-dimensional visualization and preoperative planning for operative safety. Furthermore, hepatobiliary and pancreatic navigation surgery using augmented reality for either open or laparoscopic surgery is performed at Daisan Hospital in collaboration with the Institute for High Dimensional Medical Imaging Research Center. With regard to nutritional therapy for patients with cancer, clinical and experimental studies are examining enhanced recovery after surgery, surgical site infection, and the use of eltrombopag before laparoscopic splenectomy for idiopathic thrombocytopenic purpura.

Digestive surgery (comprehensive)

We have been pursuing clinical research at 4 affiliated university hospitals. Since 2014,

we have had 6 articles published in English.

Because surgical infection is a common problem that is important to control, we have assigned members of the staff to be in charge of surgical infection at each university hospital and are encouraging the reduction of surgical infection. Three of the 4 hospitals are participating in the Japan Nosocomial Infections Surveillance program supervised by the Japanese Ministry of Health, Labour and Welfare, and 3 of the 4 hospitals are teaching-hospitals approved by the Japan Society for Surgical Infection. Although we are active in presentations at national conferences, we must publish articles other than case reports.

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Department of Surgery
Division of Thoracic Surgery and of Breast and Endocrine Surgery

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General Summary

The Divisions of Thoracic Surgery and of Breast and Endocrine Surgery were established in June 2005. Since then, we have provided comprehensive diagnostic and therapeutic services for patients with surgical diseases of the chest, breast, and endocrine system. We are leaders in our fields of surgery, with a focus on minimally invasive surgery.

Research Activities

Thoracic Surgery

Thoracic surgeons of The Jikei University connect biomedical breakthroughs to practical applications to improve lives. Clinical research is an important part of our program and can provide novel treatments before they are widely available. Our research topics include (1) pulmonary function after lung resection, (2) surgery for lung cancer with oligometastatic disease, and (3) the effect of pleural abrasion and coverage with a polyglycolic acid sheet for pneumothorax. We are also involved in multiple basic research studies to understand, diagnose, and develop new treatments for pulmonary disorders and lung cancer.

Lung transplantation has become a well-established therapy for patients with severe or terminal pulmonary diseases that cannot be cured completely by medical treatments. Bronchiolitis obliterans and bronchiolitis obliterans-related syndrome are recognized as severe complications of lung transplantation. We have hypothesized that carbon monoxide can improve bronchiolitis obliterans through the inhibition of T-cell infiltration and the downregulation of inflammatory cytokine expressions in the transplanted grafts. To assess this hypothesis, we will examine the effect of carbon monoxide with an artificial gas carrier in a mouse model of orthotopic tracheal transplant.

We are also starting our basic research in oncology. Tumors exhibit multiple somatic mutations. Neoantigens derived from such tumor-specific mutations are potential targets for antitumor immune responses. The role of neoantigens in naturally occurring and therapeutically induced immune responses to cancer. We are planning to investigate mutation burden, neoantigen load, and the depletion of expected antigenic mutations in cases of lung cancer surgically removed by our division. Our aim is to find the new neoantigens derived from tumor-specific mutations that can be targets for antitumor immune

responses.

Breast and Endocrine

1. Clinical study

1) Immediate breast reconstruction is rarely performed after neoadjuvant chemotherapy. The purpose of our clinical study is to evaluate the short- and long-term outcomes of this procedure after neoadjuvant chemotherapy in patients with breast cancer.

2) We have participated in a multicenter clinical trial to evaluate the oncological safety of patients with metastases to 1 or 2 sentinel nodes after omission of axillary lymph node dissection.

3) Aromatase inhibitors have been established as the gold-standard therapy for postmenopausal patients. We plan to participate in a multicenter clinical trial to evaluate the efficacy of denosumab for the treatment of aromatase inhibitor-associated bone loss in Japan.

4) Cryoablation with extremely cold temperatures to destroy tumor tissues has been recognized as a highly efficient cancer therapy and is used to treat several types of cancer, including those of the breast, skin, prostate, and bone. The efficacy and side effects have been evaluated in patients with early-stage breast cancer.

5) Therapeutic strategy for oligometastatic breast cancer

For 30 years we have analyzed patients with metastatic breast cancer. The analysis indicates that oligometastatic breast cancer is a distinct subgroup with a long-term prognosis superior to that of other metastatic breast cancers. We are performing prospective studies to characterize oligometastatic breast cancer and to evaluate the efficacy of a multidisciplinary strategy, including medication, radiotherapy, and resection, to improve prognosis.

6) Because of recent progress in the diagnosis and treatment of breast cancer and with the development of fertility preservation, oncofertility has become more important. With close cooperation among healthcare providers, we analyzed how we provide information of fertility preservation to young patients with breast cancer.

7) Lenvatinib, a tyrosine kinase inhibitor, prolongs the progression-free survival of patients with thyroid cancer. We have participated in a multicenter clinical trial to evaluate the antitumor effects and side effects of lenvatinib for patients with thyroid carcinomas, including papillary carcinoma, medullary carcinoma, and undifferentiated carcinoma.

2. Basic research

1) In breast cancer, cells with high expression of CD44 and with no or very low levels of CD24 are identified as cancer stem cells. Several previous studies have shown that CD44+/CD24 − /low tumor cells in primary breast cancer are prognostic factors for relapse and survival. How CD44+/CD24 − /low tumor cells in metastatic sites affect outcome has been unclear. We have analyzed the presence of CD44+/CD24 − /low tumor cells among metastatic tumor cells to identify any biological characteristics that reflect long-term survival.

2) Dual specificity tyrosine phosphorylation regulated kinase 2

Accumulating evidence suggests that dual-specificity tyrosine-regulated kinase 2 (DYRK2) functions as a tumor suppressor by regulating cell survival, differentiation, proliferation, and apoptosis. However, little is known about the mechanisms of transcrip-

tional regulation by DYRK2 in cancer progression. We found that reduced DYRK2 expression increases cyclin-dependent kinase 14 (CDK14) expression, which promotes cancer cell proliferation and invasion in vivo. Expression of CDK14 and of DYRK2 are inversely correlated in human breast cancer tissues.

3) The detection of antigens of thyroid carcinoma in sera

A monoclonal antibody, designated JT-95, was made against a thyroid papillary carcinoma obtained by the Department of Breast and Endocrine Surgery. We are attempting, in collaboration with the Molecular Cell Biology Division of The Jikei University, to measure the antigen recognized by JT-95 in the serum of patients with papillary carcinoma. The quantity of the JT-95 antigen is higher in patients with papillary carcinoma, especially those with metastasis to lung or bone, than in patients with breast carcinoma. We are now trying to improve chromatography for JT-95 to more easily detect thyroid carcinoma antigens.

Publications

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Department of Surgery
Division of Pediatric Surgery and Vascular Surgery

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General Summary

Pediatric Surgery
Surgery for children at The Jikei University Hospital is offered by a highly trained, expert team of pediatric surgical professionals who specialize in the diseases and conditions affecting young people. Our surgeons specialize in treating neonates, infants, children, and adolescents and understand their unique needs.

Vascular Surgery
Research projects of our department have focused on the development of the endovascular repair of aortic aneurysms, treatment of peripheral arterial disease with new techniques and devices including clinical trials.

Research Activities

Pediatric Surgery
1. Education
Education for medical students: Patients who undergo pediatric surgery often have a congenital anomaly. The lecture for students on pediatric surgery is based on embryology.
Education for training physicians: Three objects for training physicians in pediatric surgery are: (1) how to take blood and venous route from pediatric patients, (2) understanding blood test results and fluid therapy for pediatric patients, and (3) learning basic surgical techniques for pediatric surgery.
Education for surgical residents: They are able to act as lead surgeons or assistants for pediatric surgery.
2. Clinical study
Minimally invasive and scarless surgeries. That's how we make our mark.
1) Endoscopic treatment for vesicoureteral reflux with Deflux®
There are 3 options for managing or treating vesicoureteral reflux. We recommend the endoscopic treatment with Deflux® (Oceana Therapeutics, Ltd., Dublin, Ireland), a sterile, biodegradable gel. We have treated many patients, most of whom were treated completely without complications.
2) Laparoscopic percutaneous extraperitoneal closure for inguinal hernia: a learning curve for attending surgeons and residents
Laparoscopic percutaneous extraperitoneal closure for pediatric inguinal hernia is a simple technique in which a purse-string suture made of nonabsorbable material is placed

extraperitoneally around the hernia orifice by means of a special suture needle (Lapa-Her-Closure™, Hakko Co., Ltd., Medical Device Division, Chikuma, Nagano, Japan). Concerns have been raised about the extensive learning curve for both attending surgeons and residents to master this technique. This study assessed the difference in learning curves for the safe performance of laparoscopic percutaneous extraperitoneal closure by attending surgeons and residents.

3) The Nuss procedure aims to force the sternum forward and to hold it there with an implanted steel bar, but without making a big incision to resect the abnormal cartilage. In this procedure, the curved steel bar is placed under the sternum through small incisions on the sides of the chest. We have reported and recommended a new method of safely performing anterior mediastinal dissection using endoscopic instruments and lifting hook. The number of patients with pectus excavatum treated surgically in our department is the third highest in Japan.

3. Basic study

1) MicroRNAs transported by exosomes in body fluids as mediators of intercellular communication in human neuroblastoma

Cancer-cell communication is an important and complex process, achieved through diverse mechanisms that allow tumor cells to mold and influence their environment. Recent evidence indicates that cells communicate via the release and delivery of micro RNAs packed into tumor-released exosomes. Understanding the role and mode of action of microRNAs from tumor-released exosomes is of paramount importance in the field of cancer biomarker discovery and for the development of new biomedical applications for cancer therapeutics. (Shinsuke Ohashi, Shuichi Ashizuka, Joji Yoshizawa, Masashi Kurobe, Takao Ohki. A New Index for additional superior bar in Precuts Excavatum's Nuss Procedure. The 47th Annual Meeting of the Pacific Association of Pediatric Surgeons. Banff, Canada. May 2014.)

Vascular Surgery

1. Development of endovascular repair of thoracoabdominal aneurysms

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

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

We have developed a new minimally invasive operation for aortic arch aneurysms. After

carotid-carotid bypass surgery, if needed, is performed and stent grafts are placed, a needle is used to prick the stent graft through 1 side of a carotid artery, after which a covered stent is inserted as a branch and deployed into the stent graft (in an in-situ retrograde fashion). We have examined this retrograde in-situ branched surgery in an in-vitro study and have applied it clinically. This operation is expected to be a less invasive surgery for aortic arch aneurysms. For endovascular repair of aortic arch aneurysms we also use, after approval by an institutional review board, branched thoracic arch stent grafts that are commercially available in Europe.

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

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

We have participated in a global registry and randomized controlled trial, in which most patients were enrolled in the United States, but also in Germany and Japan. After the trial's 1-year primary endpoint was reviewed, the Zilver PTX received approval from the Japanese Pharmaceuticals and Medical Devices Agency in January 2012 and is now commercially available in Japan.

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Department of Orthopaedic Surgery

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General Summary

Basic Research

The trauma team reported on their treatment of intertrochanteric femoral fracture with bone defect using β-tricalcium phosphate (β-TCP), hyaluronic acid, and the fibroblast growth factor (FGF) 2 complex. The team developed an injectable complex that promotes synostosis of the fracture of the displaced lesser trochanter and repair of cortical bone deficiency; they applied the complex to treat intertrochanteric femoral fractures with an AO classification of 31-A2. The complex supposedly exerted its effects on synostosis of the displaced fracture of the lesser trochanter.

The knee surgery team reported a technique for reconstructing the anterior cruciate ligament which uses a rectangular retrodilator they designed. Using the newly developed technique of making bone tunnels with the rectangular retrodilator, better clinical results can be obtained in anatomical reconstruction of the anterior cruciate ligament with rectangular bone-patellar tendon-bone grafts.

Clinical Research

Our clinical practice has been divided into 10 subspecialties to cover a wide range of musculoskeletal disorders and has been managed by different specialist teams: knee joint, hip joint, spine, shoulder joint, foot surgery, trauma, osteoporosis, rheumatic diseases, and sports. All teams maintain a high level of expertise and are actively involved in scientific activities.

The spine team focused on the effectiveness of percutaneous dual sacral-alar-iliac (SAI) screw fixation for the treatment of spinal deformity in adult patients.

Two SAI screws arranged linearly on the sacrum are used for correction, and the method allows shortening of both the time of surgery and irradiation while being minimally invasive; strong fixation is usually obtained.

The foot and ankle joint team performed a 3-dimensional (3D) image analysis of hallux valgus pathology. In 2018, this team focused on the 3D shape of the metatarsals and found that internal torsion of the first metatarsals is significantly more pronounced in feet with hallux valgus than in control feet.

Through this range of clinical research activities, all teams fulfill their important roles at a clinical academic hospital, and their commitment is highly valued.

Research Activities

Results of nonsurgical treatment for complete dislocation of the acromioclavicular joint
Outcomes of conservative treatment were evaluated for 43 patients who had complete dislocation of the acromioclavicular joint. In addition, the outcomes were compared with the results of 63 patients who underwent surgical treatment. The average score at final follow-up examinations was 95 points. Calcification and osteoarthritis were observed on X-ray images from 35% of the patients; however, these findings were not correlated with clinical scores. Thirty-seven athletes returned to their previous levels of sports activities within 2 months after injury. Neither the clinical results nor the incidence of posttraumatic roentgenographic changes differed significantly between patients who were surgically treated and patients who were not.

The current activities of the hand surgery division
XIAFLEX® (Endo Pharmaceuticals, Inc., Malvern, PA, USA), an injectable collagenase preparation that digests pathological palmar fascia as a treatment for Dupuytren contracture, is available at our institution. We have used XIAFLEX® to treat many cases and have obtained good results. For this reason, surgery is performed less often for pathological fascia resection in cases of Dupuytren contracture. Furthermore, patients make favorable comments about treatment with XIAFLEX® because it is, unlike conventional surgical treatment, minimally invasive.
We reported the usefulness of dual-energy computed tomography (CT) for the diagnosis of gouty tophi in the carpal tunnel. With dual-energy CT, a target is imaged with X-ray examinations of 2 different energies. The technique of using the difference in the attenuation coefficient of each energy enables the materials to be discriminated. Dual-energy CT helps with the diagnosis of and surgical planning for atypical tophi and is extremely useful for postoperative evaluation to determine whether the lesion has been removed.

Percutaneous dual SAI screw fixation for spinal deformity in adult patients
The SAI screws are now recognized as effective anchors for long-term fixation. However, clear zone areas around SAI screws have occasionally been reported, and the technique of rod connection between the S1 vertebra and the SAI screw requires specific operative skills. Our present method is based on percutaneous insertion of 2 SAI screws on each side with a modified J-probe under X-ray fluoroscopy. In addition, the correction is strengthened by 2 SAI screws arranged linearly on the sacral bone. The duration of surgery and intraoperative irradiation can be effectively shortened. While being minimally invasive, the surgery is expected to provide strong and lasting fixation.

Clinical results of flat-tapered-wedge short stem insertion in primary total hip arthroplasty for hip dysplasia in an Asian population
Cementless femoral reconstruction in patients with hip dysplasia is challenging. We studied postoperative clinical outcomes of primary total hip arthroplasty in 257 hips using flat-tapered-wedge short femoral stems for hip dysplasia in Asian patients (postoperative follow-up period, 2 years to 6 years 11 months; mean, 4.5 years). Obtained in all patients

were favorable clinical outcomes with satisfactory functional ranges were, including radiologically confirmed biological fixation. Regarding complications, split fracture of the femoral calcar region during stem insertion did not occur, and postoperative dislocation occurred in only 1 case (0.4%). The flexibility of the flat-shaped short low-volume stem used in our study allows more accurate stem positioning in patients with hip dysplasia. Better and easier positioning might reduce fractures when sufficient and secure fixation is achieved.

Osteoporosis treatment soon after total knee arthroplasty
The use of bisphosphonates soon after total knee arthroplasty has been shown to reduce the rate of replacement, 15 years later, by half. In our cases of total knee arthroplasty, when osteoporosis treatment was applied, each drug suppressed the bone resorption, which usually exacerbates postoperatively, while maintaining bone formation without decreasing bone quality.

Anterior cruciate ligament reconstruction with an originally designed rectangular retrodilator
Good clinical results have been reported with anatomical anterior cruciate ligament reconstruction with rectangular bone-patellar tendon-bone grafts. We developed a new technique for making bone tunnels with a rectangular retrodilator. Although long-term results need to be examined, our new technique is a promising procedure without major perioperative or postoperative complications.

3D image analysis study of the hallux valgus pathology
The foot and ankle team, in collaboration with the Institute for High Dimensional Medical Imaging, has been studying the pathophysiology of hallux valgus using load position CT and a 3D analysis system. Last year, we reported instability in the intercuneiform 1-2 joint in valgus feet. This year, we focused on the 3D structure of metatarsals and found that internal torsion of the first metatarsal was significantly more pronounced in feet with hallux valgus than in control feet. However, the twist was not correlated with the severity of hallux valgus, suggesting that the twist is the cause rather than an aggravating factor of hallux valgus deformity.

Comprehensive analysis of advanced glycation end-products using liquid chromatography mass spectrometry
The accumulation of advanced glycation end-products (AGEs) in bone collagen reportedly decreases bone quality. Although more than 40 AGE structures have been identified *in vivo*, a precise determination was difficult, except for pentosidine. We established a method to quantitate the amounts of Nε-carboxymethyl-lysine, Nε-carboxyethyl-lysine, methylglyoxal-derived hydroimidazolone 1, Nω-carboxymethylarginine, and pentosidine with liquid chromatography mass spectrometry. Analysis of 182 specimens of human cancellous bone dissected during total knee replacement revealed that AGEs newly determined with our research were at levels approximately 100 times as high as that of pentosidine and showed similar tendencies of accumulation. A multiple linear regression

analysis identified the independent determinants of high AGE levels to be male sex, aging, low turnover, high HbA1c level, and obesity.

Treatment of intertrochanteric femoral fractures with bone defects using an injectable complex of β-TCP, hyaluronic acid, and FGF-2

We developed an injectable complex of β-TCP, hyaluronic acid, and FGF-2, which promotes synostosis of the fracture of a displaced lesser trochanter and repair of cortical bone deficiency. We applied the complex clinically to treat intertrochanteric femoral fractures of AO classification 31-A2 in 7 patients. The complex (combining 2 g of β-TCP granules with 60% of porosities, 2.5 ml of hyaluronic acid, and 1 mg of FGF-2) was injected, under the guidance of X-ray images, into the gap between the displaced lesser trochanter and diaphysis. An intramedullary nail was then inserted. By 12 weeks after surgery, most of β-TCP had been partially absorbed into the bone, and the trochanter part had promoted its synostosis, which was shown in all cases. The complex likely effects the synostosis of displaced fractures of the lesser trochanter.

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General Summary

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

Research Activities

Cerebrovascular diseases・Endovascular surgeries

1. Analysis of the natural history of unruptured intracranial aneurysms

Since 2003, more than 5,000 patients with intracranial aneurysms have visited our department. As a leading aneurysm treatment center in the world, we have placed a great value on a precise real-time data base of patients with aneurysms. We focused on the analysis of (1) the natural history of unruptured aneurysms, (2) risk factors associated with the rupture of aneurysms, and (3) risk factors associated with treatment.

2. Analysis of biofluid mechanics on human intracranial aneurysms using computational fluid dynamics

The main topics of our current studies include (1) development of novel parameters, (2) clarifying the relationship of hemodynamic patterns with the risk of rupture, and (3) the development of a novel software program for computational fluid dynamics dedicated to the image workstation of angiographic machines.

3. Newly developed techniques of neuroradiological imaging

Although intra-arterial 3-dimensional digital subtraction angiography (3DDSA) using an angiographic C-arm system is still the gold standard for postoperative confirmation of surgical clipping of intracranial aneurysms, intra-arterial 3DDSA requires catheterization and intra-arterial injection of a contrast medium, which adds risks and time to the surgical procedure. We propose a less invasive acquisition of 3DDSA with intravenous injection in the hybrid operating room to confirm the results of surgical clipping immediately after surgery.

Neuro PBV and iFlow (Siemens Healthcare GmbH, Erlangen, Germany) are software applications for evaluating cerebral blood flow with a cerebral angiography device. These applications are usedin patients who have had a stroke to evaluate cerebral blood flow with only a cerebral angiography apparatus before, during, and after treatment. By comparing results with those of conventional cerebral blood flow tests, the usefulness of Neuro PBV and iFlow can be assessed. Metal artifact removal is a new technique for improving the accuracy of postoperative evaluation during surgery for coil embolization by reducing metal artifact. We conduct these clinical studies in collaboration with Siemens Healthcare.

4. Development of a novel intracranial stent device for the treatment of brain aneurysms

A novel intracranial stent device for the treatment of brain aneurysm is currently under development.

A preclinical animal study is ongoing. This project is supported by a research grant from the Ministry of Economy, Trade and Industry. We are now in the final stage of consecutive experiments, and the results will be reported to the Ministry in 2016.

5. Development of a new therapy for ischemic stroke using a small animal model of cerebral infarction

Using a cerebral angiography device for animals and magnetic resonance imaging, we developed a highly reproducible small animal model of cerebral infarction. With this model, new research has begun on cerebral circulation metabolism and drug discovery.

6. Establishment of a telemedicine network utilizing a novel software program for smartphones

The telemedicine software application “Join” has recently become available for any smartphone users. This application allows every member of the medical staff to have instant access to the picture archiving and communication system in The Jikei University Hospital and to communicate with an online bulletin-board system. The application is released in collaboration with NTT Docomo, which is Japan’s largest mobile service provider, serving more than 60 million customers.

Brain tumor

1. Immunotherapy against malignant glioma

We have started a clinical trial of immunotherapy with fusions of glioma cells, glioma-initiating cells, and dendritic cells. Although several cell types can induce an antitumor immune response, this function is performed most efficiently by professional antigen-presenting cells, of which dendritic cells are the most potent. We have earlier shown that immunotherapy with fusion cells made of dendritic cells and glioma cells induces safe, tumor-specific immune responses in patients with glioma. In our recent study, we have found that transfection of Poly(I:C) and interleukin (IL)-10-small interfering (si) RNA in fusion cells accelerates endogenous IL-12 secretion. The IL-12-secretory fusion cells induced a potent antitumor immune response. Therefore, we are now using Poly(I:C)/IL-10-siRNA transfected fusion cells as a tumor vaccine in clinical trial.

2. Analysis of gene mutation-associated neoantigens via a next-generation sequencer

We have previously reported that fusion cell immunotherapy, a vaccination with fusions of autologous dendritic cells and tumor cells, significantly prolongs progression-free sur-

vival and overall survival in patients with malignant gliomas. The gene expression of the malignant glioma cells used to generate a dendritic/tumor fusion vaccine was investigated to identify genes associated with the clinical responses. The number of candidate neoantigen peptides binding to HLA-A*24:02 in malignant glioma cells did not differ significantly between the effective and ineffective groups. Although 12 types of common neoantigen peptide were identified in the malignant glioma cells from the effective group, they were also expressed in the malignant glioma cells from the ineffective group.

3. Study of intraoperative imaging with C-arm computed tomography

We use a C-arm computed tomographic scanner, the *syngo* DynaCT system (Siemens Healthcare), and an image-analyzing software program to reduce metallic artifacts during the surgical resection of brain tumors. Intraoperative imaging with this system increases the resection ratio of tumors, with a surgical navigation system and a photodynamic diagnosis by 5-aminolevulinic acid. This study is aimed at establishing safe technical innovations in the surgical treatment of brain tumors.

Neurotrauma

Few institutions have performed research in neurotraumatology. A unique aspect of our department is that we have undertaken 3 major studies in this area of research. We examined the prevalence of sports-related head injury in collaboration with the Japan Society of Clinical Sports Medicine and the Japan Society of Neurotraumatology. We have also examined sports-related concussions and performed mechanical studies of head injuries through simulations. We work with Hosei University for studies of American football, with the Japan Football Association for studies of football, and the Japan Boxing Commission for studies of boxing.

Spine and Syringomyelia

Each year approximately 30 patients with syringomyelia are treated surgically in our department. To date, we have treated more than 750 patients with syringomyelia. By evaluating cerebrospinal fluid (CSF) obstruction at the craniovertebral junction in patients with syringomyelia related to Chiari malformation, the relation of CSF circulation blockage to cavitation of the spinal cord has been clarified. Therefore, improving CSF circulation becomes the goal of surgical treatment. However, the mechanism of cavitation of the spinal cord is not fully understood. In patients with Chiari malformation, the cerebellar tonsils and the ventral vector (i.e., dens) compress the spinal cord and restrict CSF circulation. We examined whether these 2 factors influence the effects of foramen magnum decompression.

We also developed an implant for cervical laminoplasty. The use of this implant corresponds with various surgical methods, and its initial fixation power has increased. Furthermore, we have performed spinal surgery in a hybrid operating room as a global pioneer, making the procedure safer and more reliable for patients.

We have presented these research results at the Neurospinal Society of Japan, the Japan Neurosurgical Society, and the Global Spine Congress.

Division of Pediatric Neurosurgery

In the division of pediatric neurosurgery, we offer gentle and minimally invasive operations to many patients with spina bifida, hydrocephalus, cranial facial anomaly, and brain tumor. We also follow-up postoperative patients and inoperative patients with diseases to assess their development and conditions for long periods of treatment in the outpatient clinic.

Over the past 15 years, new cases in various entities number more than 2,000. This division currently consists of a consultant, a division staff, and a resident, promoting clinical research through various clinical activities.

As for spina bifida, we are currently examining the prognosis of neurological functions by operating under neuromonitoring and examining an early detection system for occult spina bifida through the type of skin stigmata.

We are also developing operative procedures and instruments for hydrocephalus, intracranial cysts, and brain tumor by neuroendoscopic maneuvering and are proposing the usage of navigation systems.

We have proposed an age-related operative method for craniofacial surgery and have won awards in Japan and international societies of pediatric neurosurgery.

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Department of Plastic and Reconstructive Surgery

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General Summary

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

Research Activities

Introducing the techniques of aesthetic surgery in open septorhinoplasty
Rhinoplasty plays a great role in the treatment of nasal obstruction, because the anterior nasal airway is responsible for 70% of airway resistance. Caudal septal deviation was known to cause nasal obstruction but had rarely been treated in Japan because the caudal septum is a key structure to be preserved in conventional intranasal septoplasty. Damage to the caudal septum may compromise the shape of the nasal pyramid. We have recently collaborated with otorhinolaryngological surgeons in performing functional rhinoplasty and have introduced open septorhinoplasty techniques that are widely used in aesthetic surgery. The open approach allows the deviated L-strut to be corrected under direct vision and is best indicated for treating caudal septal deviation and internal/external nasal valve obstruction.

Treatment of nasal valve obstruction
The nasal valve region plays a key role in nasal breathing. Although a variety of techniques for treating nasal valve compromise have been described in the international literature, they are rarely used in Japan. Both nostrils collapse completely under forced inspiration owing to weak cartilagenous support. Other than narrowing of both nostrils, no nasal deformity was present. Preoperative computed tomography (CT) revealed that the nasal septum was straight and that the inferior turbinate was not swollen. Anterior nasomanometry showed that nasal resistance in the sitting position was increased. Open septorhinoplasty was performed, and a 10-mm-wide L-strut was left intact. The internal nasal valve was widened with a pair of spreader grafts. The external nasal valve was reinforced with a columellar strut and an alar batten graft. The spreader graft was given the role of a sep-

tal extension graft to support the tip of the nose. Postoperative nasal resistance was less than the standard for adults, and the nostrils never collapsed under forced inspiration. Nasal valve compromise can cause nasal obstruction, even when the septum is straight, but can easily be treated with techniques well known in aesthetic surgery.

A new "J septoplasty" technique for correction of mild caudal septal deviation

A major drawback of the Killian incision is the inability to access the caudal septum and correct caudal septal deviation. Open and hemitransfixion septorhinoplasty are considered necessary in such cases. We developed a new septoplasty method named "J septoplasty" after Dr. Jiro Iimura, which can be successfully used for patients with mild caudal septal deviation. In this study, we evaluated the outcome of this technique.

Methods: We prospectively collected data of 16 patients with mild caudal septal deviation who had undergone endoscopic septoplasty from November 2015 through October 2017. A modified Killian incision was made on the concave side of the septum. The central part of the cartilage was preserved, and excess cartilage was resected; the central part of the cartilage was sutured to the caudal cartilage.

Results: Postoperatively, the ratio of the area of the convex side to that of the concave side in the anterior portion of the nasal cavity was significantly improved, as revealed with CT analysis ($p < 0.001$). Nasal obstruction was significantly reduced or eliminated in all patients ($p < 0.001$).

Conclusion: The J septoplasty method for the correction of mild caudal septal deviation is easy to perform through a modified Killian incision and seems to be useful in selected cases.

Ilizarov minifixator

The Ilizarov minifixator is a useful device in various areas of hand surgery. Its clinical usefulness was demonstrated in the treatment of fractures (open and comminuted fractures and fractures adjacent to the joint), malunion of fractures, and pathological fractures caused by enchondroma and joint contractures. This device has also been used with good results in bone lengthening and the temporary traction of joints. Use of the Ilizarov minifixator is an effective and noninvasive method and is highly recommended for selected cases.

Surgical strategy for Apert syndrome: Retrospective study of developmental quotient and 3‐dimensional CT

Many surgical techniques have been used to treat craniosynostosis. However, the indications for and timing of surgery remain unclarified. Most of the skull growth in children with craniosynostosis is completed in the first year of life, and the bone is strong enough to undergo distraction osteogenesis. This study aimed to consider the best timing for cranial expansion and surgical strategy for Apert syndrome. From January 2002 through December 2011, 13 patients with Apert syndrome underwent surgery and were followed up for more than 5 years. The primary surgery was fronto‐orbital advancement. We evaluated the postoperative developmental quotient every year and evaluated cosmetic change with 3‐dimensional CT when the patient was older than 5 years. Eleven of 13 patients had

improved developmental quotient scores, with no significant intergroup differences. Three-dimensional CT evaluation showed cases with remnant brachycephalic deformity in both groups. Two patients with remnant plagiocephalic deformities tended to have primary surgery earlier in life than did other patients. Thus, primary surgery had little effect on psychological development when delayed and can be delayed unless the intracranial pressure must be controlled. We conclude that fronto-orbital advancement cannot sufficiently improve the brachycephalic appearance; other procedures, such as posterior vault distraction, might be better alternatives.

A simple way to measure glucose and lactate values during free flap head and neck reconstruction surgery

Evaluation of flap blood flow is necessary to detect abnormalities and perform salvage surgery. This study determined whether intraflap blood glucose and lactate values measured with a simple instrument can detect impaired blood flow during head and neck reconstruction. We prospectively analyzed 82 cases of head and neck cancer; blood flow was impeded after reconstruction in 74 cases. Levels of glucose and lactate were regularly measured over a period of 48 hours, from the time of flap elevation, as predictor variables. Blood flow obstruction was the outcome variable. Other study variables included primary site, flap type, sex, age at operation, height, weight, body mass index, the presence or absence of diabetes, ischemia time, and operative time. Logistic analysis, using glucose and lactate values at the time of blood flow failure, was performed. Cutoff values were calculated with a receiver operating characteristic analysis. The choice of the flaps was as follows: 20 free jejunum flaps, 19 anterolateral thigh flaps, 12 fibular flaps, 11 radial forearm flaps, 8 rectus abdominis myocutaneous flaps, and 4 other flaps. Congestion was observed in 8 of 82 flaps, including 3 anterolateral thigh flaps, 3 radial forearm flaps, 1 free jejunum flap, and 1 rectus abdominis myocutaneous flap. The intraflap blood glucose values in the normally progressing cases gradually decreased until 16 hours postoperatively and thereafter recovered to normal levels. Intraflap blood lactate values were increased until 8 hours postoperatively and subsequently decreased. The odds ratio during congestion was significantly different for only lactate (odds ratio, 2.55; P = .014), and the cutoff values were 4.2 mmol/L for sensitivity and 6.7 mmol/L for specificity. Intraflap blood glucose and lactate values might reflect the transition of the postoperative circulation of free flaps. During congestion, lactate values change more sensitively than do blood glucose values.

Publications

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Department of Cardiovascular Surgery

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General Summary

The major achievements in our department include both clinical and experimental animal studies. The clinical studies include those establishing excellent surgical performance, investigating new techniques, and evaluating alterations in cardiac performance and long-term results after cardiac surgery. In addition, analysis based on the Japanese cardiovascular surgery database is becoming new projects. We are also continuously performing several in-vivo and in-vitro experimental studies. The major activities in congenital and adult sections are described below.

Research Activities

1) Postoperative geometry of left atrioventricular valve in atrioventricular septal defect: Echocardiographic assessment was performed in 14 patients who underwent intracardiac repair to identify the risk profile of LAVV regurgitation.
2) Clinical benefits of blood cardioplegia (BCP) during a pediatric heart surgery: Biochemical markers for myocardial injury (troponine T) and oxidative stress (8-isoprostane) were evaluated to show the role of BCP.
3) Experimental study on myocardial protection by a single dose Del Nido cardioplegia was performed and its safe ischemic time was demonstrated to be 90 min.
4) The cardiac conduction system (CS) in human heart specimens with various types of cardiac anomaly was visualized by the high-resolution phase contrast CT imaging, and 3D reconstruction of CS was feasible.
5) A quantitative assessment of pulmonary vasculature was performed by the newly developed index, the total pulmonary vascular volume index, using computed tomography.
6) Toward improvement of the clinical outcomes after aortic valvuloplasty surgery:
The majority of surgical intervention for aortic regurgitation (AR) had been aortic valve replacement (AVR) using a prosthetic valve. However, the majority of the patients with AR is relatively young, thus bleeding and thromboembolic events caused by life-long anticoagulation therapy for mechanical prosthesis have emerged as serious concern. On the other hand, biological prosthesis has lower durability, thus repeat surgeries have emerged as another concern, although anticoagulation therapy can be eliminated. Aortic valvuloplasty (AVP) can eliminate both concerns, however, long-term durability has still been unclear, therefore durable operative procedures should promptly be established. We

have performed 19 AVPs since June 2018 and achieved good early outcomes. We have also performed experimental studies with Tokyo Women's Medical University, Institute of Advanced Biomedical Engineering and Science (TWIns) and will continue this project from now on.

7) The impact of glutaraldehyde used for mitral valvuloplasty in patients with mitral infective endocarditis on mid-term outcomes:

We have directly, locally used glutaraldehyde in mitral valvuloplasty for mitral infective endocarditis (IE) since 2004 to clarify repair area and to stabilize anastomosis site. We could have preserved the mitral valve of 30 patients (86%) out of 35 with mitral IE. We have analyzed mid-term outcomes of these 30 cases. We have experienced only one in-hospital mortality. Cumulative survival rate was 89±6% with mean follow-up period of 4.3±3.7 years and 100% follow-up rate of operative survivors. Only one case underwent mitral valve replacement due to recurrent mitral regurgitation on two months postoperatively, although infection was well controlled. Other 29 patients underwent no reoperation. Nakamura published these results as an article entitled "Safe use of glutaraldehyde to repair the destroyed valve in active infective mitral valve endocarditis (Circ J. 2018; 82: 2530-4)". Kunihara published an editorial comment for this article entitled "Fixation or disinfection? (Circ J. 2018; 82: 2472-4)".

8) Anatomical research for accurate tricuspid annuloplasty:

Tricuspid annuloplasty has widely been performed as a simple and safe procedure and several annuloplasty rings have been available from multiple manufacturers. Each annuloplasty ring is designed based on normal anatomy of the tricuspid valve, however, the detail of the background data has never been published. Therefore, designs of these rings are different from normal tricuspid valve geometry measured by 3D echocardiography. Commissural markers are indicated as points on an annuloplasty ring and their location and interval are not constant among each manufacturer. The tricuspid valve has a wide variety including the additional fourth or fifth leaflet and it is still controversial where each commissure should be fixed.

9) We have performed total arch replacement (TAR) for patients with the atherosclerotic aorta at high risk of embolic stroke. There was no difference in the incidence of stroke between with and without concomitant operation or between various operative procedures. Frozen elephant trunk technique is regarded as a risk factor for stroke in cases with the severely atherosclerotic aorta.

10) Registry study of early outcomes after valve-sparing root replacement and composite valved-graft replacement for aortic regurgitation.

11) Investigating the mechanism of inner mitochondrial collapsing by acute overstretch, and the mechanism of heart failure by acute volume overload:

Acute overstretch of an isolated rat papillary muscle caused inner mitochondrial collapsing with preserved sarcomere structure. Abrupt disruption in mitochondria structure by acute diastolic overstretch could account for the mechanisms on pathogenesis of acute volume-overloaded heart failure.

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Department of Obstetrics and Gynecology

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General Summary

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

Research Activities

Gynecology

1. Application of artificial intelligence for preoperative diagnostic and prognostic prediction, on the basis of blood biomarkers, in cases of epithelial ovarian cancer

A total of 334 patients with epithelial ovarian cancer (EOC) and 101 patients with benign ovarian tumors were randomly assigned to "training" and "test" cohorts. Seven supervised machine learning classifiers were used to derive diagnostic and prognostic information from 32 variables commonly available from pretreatment peripheral blood tests and age.

Ensemble methods combining weak decision trees showed the best performance in EOC prediction. For segregating EOC from benign ovarian tumors with Random Forest, the values for the highest accuracy was 92.4% and the area under the curve was 0.968. Unsupervised clustering analysis identified subgroups among patients with early-stage EOC and significantly worse survival. Machine learning systems can provide critical diagnostic and prognostic prediction for patients with EOC, and the use of predictive algorithms might facilitate personalized treatment options through pretreatment stratification of patients.

2. Identification of novel gene related to ovarian clear cell carcinoma proliferation with genome-scale CRISPR-Cas9 screens

By using genome-scale CRISPR-Cas9 screens against 4 ovarian clear cell carcinoma (OCCC) cell lines, we identified 1 candidate gene postulated to be involved in the growth of OCCC with mutations in both AT-rich interactive domain 1A (*ARID1A*) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*). While the viability of cell lines with these mutations was reduced upon small interfering RNA-mediated knockdown of the candidate gene, this knockdown did not cause any significant changes in gene expression. This result leads us to postulate that the candidate gene

induces apoptosis in a manner not yet characterized in the literature. To that end, we are now focusing on changes in cell metabolites and protein function upon knockdown of the candidate gene.

3. MicroRNA-34a as a new therapeutic target for ovarian cancer

MicroRNA-34a, which shows tumor-suppressive effects on several types of cancer, has been reported to be downregulated in ovarian high-grade serous carcinoma. In our present study, we aim to clarify the biology of microRNA-34a expression in high-grade serous carcinoma and the therapeutic viability of the microRNA on ovarian cancer.

4. Profiling of clinicopathological factors and actionable mutations with cervical cancer in the Japanese population

To elucidate the frequency of actionable genomic alterations in cervical cancers from Japanese patients, we performed the targeted sequencing for hotspot mutations in 50 cancer-related genes. Genomic DNAs extracted from formalin-fixed and paraffin-embedded tumor tissues were subjected to the analysis. Copy number alterations were detected with the TaqMan real-time quantitative polymerase chain reaction (PCR) assay in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA)*, erb-b2 receptor tyrosine kinase 2 (*ERBB2*), phosphatase and tensin homologue (*PTEN*), and serine/threonine kinase 11 (*STK11*). Human papillomavirus infection was confirmed with genetic testing and the in-situ hybridization method. The oncogenic or pathogenic variants registered in the OncoKB and ClinVar databases were defined as the actionable mutations in this study. We are processing the correlation analysis between genomic alteration, clinicopathological factors, and prognosis.

5. Invariant natural killer T cells are a unique subset that share properties of both T cells and natural killer cells

Invariant natural killer T cells (iNKT cells) express a semi-invariant T cell receptor that can interact with a glycolipid presenting molecule called CD1d. Upon activation with lipid antigen, iNKT cells can exert direct anticancer activity, behaving like CD8 T cells, and indirect anticancer activity through production of many cytokines. Our laboratory developed a new glycolipid named 7DW8-5, which showed higher affinity to CD1d and a stronger effect as an malaria vaccine adjuvant than does alpha-galactosylceramide. Therefore, we are investigating the antitumor effects of 7DW8-5 as iNKT cell-targeting immune therapy.

6. Development of a new diagnostic tool for OCCC using a cell-free circulating DNA detection method

We attempted to detect mutations by means of droplet digital PCR (ddPCR) in the genes *PIK3CA* and Kirsten rat sarcoma viral oncogene homolog (*KRAS*) in cell-free (cf) DNA from patients who have OCCC. We were able to specifically detect *PIK3CA* mutation H1047R and *KRAS* point mutation G12D in cfDNA from patients with OCCC and monitor their response to therapy. Our results suggest that detection via ddPCR of mutations in cfDNA would be useful for diagnosing OCCC and predicting its recurrence.

7. Impact of mismatch repair protein and PD-L1 expression for therapeutic stratification in Japanese ovarian clear cell carcinoma

The study cohort comprised 113 patients with OCCC treated at a single institution. Protein expression levels in ARID1A, MLH1, PMS2, MSH2, MSH6, and PD-L1 were evalu-

ated by immunohistochemistry (IHC). MMR proteins disappeared in two cases (1.8%), specifically those who had synchronous double cancer with endometrial carcinoma and a family history of Lynch syndrome-related tumor. PD-L1 expression was diffused in 17 cases (15.0%). The diffused PD-L1 expression was significantly associated with ARID1A expression loss ($p = 0.003$), but no other correlations existed between PD-L1 expression and clinical parameters including prognosis. Although only few Japanese OCCC cases showed MMR deficiency as evaluated by IHC, immune checkpoint signals were activated even in MMR-intact OCCC, possibly through ARID1A interaction.

Perinatology

1. Amplicon-sequencing-based noninvasive fetal genotyping for *RHD*-positive D antigen-negative alleles

Fetal *RHD* genotyping via cfDNA might prevent unnecessary antibody application. We developed an amplicon-sequencing method that can estimate the type of paternally inherited fetal *RHD* allele from 4 major *RHD* alleles in the Japanese population. This method correctly determines the RhD blood type of the fetus even when RhD-negative pregnant women possess an *RHD*-positive-D antigen-negative allele. Because of its high accuracy and reasonable cost, this method is considered the first reliable noninvasive fetal *RHD* genotyping method for Japanese and other East Asian populations.

2. Retinoid acid-induced placental vascular hypoplasia in rats

Retinoic acid, a vitamin A derivative, has been suggested to be associated with preeclampsia. Therefore, we compared the expression of retinoic acid receptors and responders in the placentas of pregnant women with preeclampsia or with a normal pregnancy. Retinoid receptor expression was observed in the placenta of patients with pregnant hypertensive nephropathy as compared to that of patients with a normal pregnancy.

3. Development of novel cell therapy for fetal hypophosphatasia

The aim of our research is to develop a novel fetal therapy for hypophosphatasia. We established a mouse model of hypophosphatasia and are developing an alkaline phosphatase-expressing cell line. This study will be supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science in 2019.

4. Genomics and epigenetics research in perinatal region

The following studies were performed to develop methods for extracting targeted genomic/epigenomic information from crudely mixed genomic/epigenomic information.

a. Single-cell DNA sequencing of fetal cells in maternal peripheral blood for noninvasive prenatal diagnosis

b. The possibility of using placenta-specific interindividual differences in genome-wide DNA methylation profiles to assess intrauterine environments

c. Investigation via whole-genome single nucleotide polymorphism arrays of novel candidate genetic factors causing recurrent abortions in Japanese women

d. Genetic/epigenetic analyses for undiagnosed and rare perinatal diseases

5. Autonomous trisomic rescue of Down syndrome cells

In our study, we continuously cultivated induced pluripotent stem cells (iPSCs) with chromosome 21 trisomy and unexpectedly obtained revertant cells with normal chromosome 21 diploids from the trisomic cells. Repeated experiments revealed that this trisomy res-

cue was not due to mosaicism of chromosome 21 diploid cells and had occurred at an extremely high frequency. We herewith report the spontaneous correction from chromosome 21 trisomy to disomy without genetic manipulation, chemical treatment, or exposure to irradiation. The revertant diploid cells will possibly serve as a reference for drug screening and a raw material of regenerative medicinal products for cell-based therapy.

Reproductive endocrinology

1. The effect on fertility of molecularly targeted chemotherapies

We are studying the effects on ovaries of novel anticancer drugs, such as molecularly targeted drugs. Molecularly targeted drugs target specific molecules to suppress the increase of cancer and are said to have few side effects. However, their effect on ovaries is unknown. We are assessing the effects on ovaries of several molecularly targeted drugs.

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Department of Urology

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General Summary

We performed both basic and clinical research in the following areas: oncology, involving such sites as the kidney, bladder, prostate and testes; anatomy, physiology, and pharmacology of the bladder and urethra; imaging and radiology; infections and inflammation of the genitourinary tract, such as interstitial cystitis and prostatitis; infertility; andrology and sexual function; urolithiases; technology and instruments, such as laparoscopy; transplantation; neurourology; and female urology, such as benign prostatic hyperplasia, overactive bladder, neurogenic bladder, stress urinary incontinence, and pelvic floor prolapse.

Research Activities

Basic research
We performed several studies to clarify the biology of urological malignancies, the mechanisms of voiding, and the pathophysiology of interstitial cystitis. Most studies have been presented at the annual meetings of the Japanese Urological Association and the American Urological Association. These projects are as follows.
1. Establishment and biological analysis of our new prostate cancer model, named JDCaP, derived from a Japanese patient
2. Study of the incidence of latent prostate cancer
3. Analysis of circulating tumor cells in castration-resistant prostate cancer
4. Stem cell therapy for interstitial cystitis, stress urinary incontinence, and underactive bladder

Clinical research
Several clinical studies have been performed in our institution. The results of several studies have already been reported at the annual meetings of the Japanese Urological Association and the American Urological Association.
1. Prospective study of the efficacy of the sacral epidural block versus the pelvic plexus block for transrectal prostate needle biopsy
2. Clinical study of sentinel lymph-node dissection for prostate cancer and bladder cancer
3. Analyses of urine markers, including cytokines, chemokines, and growth factors, in patients with interstitial cystitis

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Department of Ophthalmology

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General Summary

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

Research Activities

Cataract
We are able to choose various premium intraocular lenses (IOLs), such as multifocal, toric, and yellow IOLs. We implant these new IOLs through microincisions and evaluate subsequent visual function.

Neuro-ophthalmology
1. We report an unusual case of spontaneous improvement of visual acuity in a boy with neuromyelitis optica spectrum disorder. Ophthalmic and MRI evaluations demonstrated the presence of unilateral optic neuritis. After serological tests showed positivity for anti-aquaporin 4 antibody, neuromyelitis optica spectrum disorder was diagnosed. Because the patient's unilateral optic neuritis was considered to reflect mild disease activity, only follow-up observations were performed. One week after the first examination, both visual acuity and central scotoma had improved. In the absence of any specific treatments, good visual acuity has remained for 20 months, with no relapse of optic neuritis.
2. We reviewed the differential diagnosis of visual impairment of sudden onset and the management of optic neuritis.
3. In a lecture on the pathophysiology of the clinical aspects of optic neuritis we reported on the polymorphism in the promoter region of aquaporin 4 associated with an increased risk of neuromyelitis optica in the Japanese population and on 2 cases of the coexistence of neuromyelitis optica and myasthenia gravis. We lectured on recent topics of drug-induced optic neuropathy caused by novel drugs, including targeted agents in cancer therapy, immunosuppressive agents, and biological agents.

4. We reported the characteristics of Japanese patients with Leber's hereditary optic neuropathy and a trial of the drug idebenone, a case of immunoglobulin G4-related infiltrative optic neuropathy with immunoglobulin G4-related sinusitis, and MRI findings of nontraumatic orbital subperiosteal hemorrhage.

Ocular oncology and histopathology
1. We reported on a case of orbital cavernous hemangioma removed using a navigation system and on 2 cases of lacrimal sac tumors found with lacrimal endoscopy.
2. We reported the visual and vital prognosis of 36 patients with optic glioma treated with chemotherapy alone. The overall 10-year survival rate was more than 90%. We reported cases misdiagnosed as optic glioma, i.e., infiltrating optic neuropathy with the recurrence of acute lymphocytic leukemia, hemangioma in the optic nerve with von Hippel-Lindau disease, and medulloepithelioma in the optic chiasma.

Glaucoma
Analysis with the Markov model of the effects of an examination program showed that glaucoma produces an irreversible visual field loss and the most common type of visual impairment in Japan. Early detection and treatment until the advanced stage are important because symptoms are poor. We used the Markov model to analyze the effects of screening for glaucoma in adults. The early detection and early treatment of glaucoma are economically beneficial.

Functional neuroimaging
Cortical myelination was calculated with T1-weighted images divided by T2-weighted images as cortical myelin mapping with clinical MRI. In patients with hemianopsia and altered optic radiation, myelin content was reduced, particularly in the posterior portion of the primary visual cortex, but was better conserved in the anterior portion, respecting their visual field defects.

Developmental functional abnormality
Diffusion tensor imaging was performed to evaluate axonal-axonal density by means of fractional anisotropy on major white-matter tracts to compare subjects with or without strabismus. The fractional anisotropy value of the subjects with strabismus was reduced at the forceps major, which connects the occipital lobes via the splenium of corpus callosum.

Visual neuropsychology
With the use of functional MRI or diffusion MRI or both, many eye diseases have been shown to change the visual cortex and the visual tract. We are now attempting to stabilize a scanning procedure for quantitative MRI and to apply it to a volunteer who has an eye disease. Quantitative MRI allows us to directly measure T1 values. By using T1 values, we can estimate cell compositions at a voxel, each of which is an array of elements in a brain image.

Low vision
We assessed the effect of rehabilitation for patients with visual field loss by using the active field analyzer, which can help clarify a visual search function that is a factor in the specificity of the visual field but not in visual acuity.

Vitreoretinal surgery
We have used a 23-, 25- and 27-gauge transconjunctival vitrectomy system for the treatment of macular hole, epiretinal membrane, macular edema, and rhegmatogenous retinal detachment. The 25- and 23-gauge sutureless vitrectomy techniques decrease the surgical trauma and improve patients' postoperative comfort. The 25- and 23-gauge instrumentation is effective for a variety of vitreoretinal surgical indications. Although the infusion and aspiration rates of the 25- and 23-gauge instruments are lower than those for the 20-gauge high-speed vitrectomy system, the use of a 25- and 23-gauge transconjunctival vitrectomy system might effectively reduce operative times of select cases that do not require the full capability of conventional vitrectomy.
To evaluate the clinical efficacy of a 7-mm intraocular lens (Eternity®, Santen Pharmaceutical Co., Ltd.) for combined pars plana vitrectomy, phacoemulsification, and intraocular lens implantation, we observed the visibility of the retina during vitrectomy and measured the depth of anterior chamber preoperatively and postoperatively with the Pentacam® camera system (Oculus Optikgeräte GmbH).
We will evaluate the changes in regular and irregular corneal astigmatism after 25-gauge and 23-gauge transconjunctival sutureless vitrectomy.
We investigated changes in corneal thickness following vitreous surgery and determined whether such changes can be used as criteria for evaluating the invasiveness of vitrectomy.
To treat a lens nucleus that dropped during cataract surgery, we removed the nucleus through the corneal wound without using a pars plana vitrectomy.

Electrophysiology
We are recording electroretinograms to evaluate possible functional disorders at the retinal-cell level in hereditary retinopathy, retinal dystrophy, and macular disease. The electroretinographic waveforms are compounded from the responses of various retinal cells, such as ganglion, amacrine, bipolar, and photoreceptor cells, which are recorded as a single wave pattern.

Diabetic Retinopathy section
A group of vulnerable retina ganglion cells have been reported in patients with diabetes mellitus and in animal models of diabetes. We are recording electroretinograms to evaluate retinal function in patients with diabetes but without retinopathy, as shown with ophthalmoscopy.

Uveitis
We reported on a patient with an atypical presentation of a phakic IOL who initially had vitelliform submaculopathy, a vitreous haze, and a peripheral retinal focus. We described

detailed enface imaging of swept-source optical coherence tomography findings for 3 patients with acute zonal occult outer retinopathy.

Macular degeneration
We reported the effects of photodynamic therapy plus intravitreal aflibercept with subtenon triamcinolone acetonide injections for treating aflibercept-resistant polypoidal choroidal vasculopathy. Triple therapy improved visual and anatomical outcomes in patients who had polypoidal choroidal vasculopathy with recurrent or resistant retinal fluid and pigment epithelial detachment after multiple injections of intravitreal aflibercept.

Biochemistry
We examined the role of chemokines in an ATP-binding cassette, sub-family A, member 4 (*Abca4*)$^{-/-}$ retinol dehydrogenase 8 (*Rdh8*)$^{-/-}$ mouse model of Stargardt disease and the MER proto-oncogene tyrosine kinase (Mertk)$^{-/-}$ mouse model of retinitis pigmentosa. Our results indicated that the chemokine (C-C motif) ligand 3 gene (*Ccl3*) plays an essential role in regulating the severity of retinal inflammation and degeneration in these mouse models.

Color vision defects and genetic analysis of retinal diseases
1. Retinitis pigmentosa and its allied disorders have genetic heterogeneity. To identify pathogenic variants, we performed direct sequencing and whole-exome sequencing analysis for these disorders and successfully identified several novel pathogenic variants. In addition, among cases of congenital color blindness, we analyzed genetic variations for congenital achromatopsia, including congenital achromatopsia and blue cone monochromacy.

Cornea
We will assess the age and disease condition of patients with keratoconus and determine the most appropriate approach for improving vision and quality of life.

Oculoplastic
1. We reported the effect of fatty degeneration of the levator palpebrae superioris muscle on surgical outcomes for involutional blepharoptosis
2. We reported differences in surgical outcomes between anterior and posterior approaches for blepharoptosis repair
3. We lectured on utility in endonasal dacryocystorhinostomy using a navigation system

Publications

Shoji N*[1]*, Arakaki Y*[2]*, Nakamoto K*[3]*, Yamamoto T*[4]*, Kuwayama Y*[5]; Collaborative Bleb-related Infection Incidence and Treatment Study Group (Noro T) (*[1]***Kitasato Univ Hosp,** [2]***Univ the Ryukyus,** [3]***Tokyo Metropolitan Police Hosp,** [4]***Gifu Univ Graduate Sch Med,** [5]***Osaka Koseinenkin Hosp).*** Efficacy of predetermined therapeutic measures against bleb-related infection in the Collaborative Bleb-related Infection Incidence and Treatment Study. *Acta Ophthalmol.* 2018 Mar; **96:** e229-36.

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Department of Otorhinolaryngology

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General Summary

We have 5 research areas, which are otology, rhinology, laryngology, head and neck surgery, and sleep science. The researchers in these areas worked on developing safe and effective surgical techniques. They also do basic research in their specialized fields and have achieved excellent results.

Research Activities

Research issues in otology

Our research projects span experiments on the fundamental aspects of middle ear mucosa regeneration and its clinical application, research on gene therapy targeting epithelium with residual cholesteatoma, and the development of a navigation system utilizing virtual-reality technology to increase the safety of surgery. In addition, cases of cholesteatoma surgery performed at our hospital are recorded in our database, which is used to analyze the condition of patients, to select operative methods, and to review postoperative outcomes. We perform approximately 300 middle ear surgeries annually at our hospital. Cochlear implantations performed every year have also yielded favorable results. We perform skull-base surgery, including that for cholesteatoma in the petrous part of the temporal bone, in conjunction with the Department of Neurosurgery, and have found that hearing and facial nerve function can be preserved in many cases. We also perform acoustic tumor surgery. For secretory otitis media we select the treatment method in individual patients depending on the degree of development of the mastoid air cells. In the field of neuro-otology, we have introduced vestibular evoked myogenic potential (VEMP) testing to evaluate saccular function in patients with such conditions as vestibular neuritis, Meniere's disease, and dizziness of unknown cause to facilitate diagnosis and treatment. Moreover, we are examining the prevalence of abnormal saccules in various disorders as measured with VEMP testing, the ictal and nonictal phases of Meniere's disease, and the incidence of VEMP abnormalities according to disease stage. We also adopted the video head impulse test (vHIT) for examining the function of the semicircular canal.

Research in rhinology

We are analyzing data on factors related to the intractability of rhinosinusitis obtained from patients undergoing endoscopic sinus surgery (ESS) and from prospective studies of

the postoperative course. We perform special care for skull-base diseases, such as pituitary tumors and cerebrospinal fluid leak, with a good relationship with the Department of Neurosurgery. In an attempt to expand the indications for ESS from paranasal sinus tumors to skull-base surgery, including that for spinal fluid leakage, skull-base tumors, and pituitary gland tumors, and to improve the safety of ESS, we have performed high-technology navigation surgery in which 3-dimensional endoscopic images and stereonavigation images are superimposed. We have planned clinical studies and developed treatment methods for patients with a variety of olfactory disorders. To clarify the pathogeneses of eosinophilic chronic rhinosinusitis and allergic fungal rhinosinusitis, we investigate how environment fungi and bacteria induce activation and degranulation of human eosinophils and the airway epithelium.

Research on head and neck tumors

For common advanced cancers we perform radical surgery (e.g., total pharyngolaryngectomy combined with reconstruction by means of free intestinal flap transfer for hypopharyngeal cancer and total laryngectomy for laryngeal cancer); however, we perform larynx-preserving surgery (partial hypopharyngectomy combined with reconstruction by means of free-flap transfer and partial laryngectomy) to preserve function, especially vocal function, to the greatest extent possible. We have obtained favorable outcomes in terms of both laryngeal preservation and survival. For conservative therapy and postoperative treatment for advanced cancer, we perform radiotherapy, alone or with concurrent chemotherapy with cisplatin and fluorouracil, and have obtained favorable results. In regard to research on cancer, we are performing basic studies and applying their findings to future studies and to clinical practice. Such fundamental studies include extraction of DNA from specimens obtained during surgery, the evaluation of epidermal growth factor receptor expression, and targets for molecularly targeted agents, such as the expression of human papilloma virus, which has been implicated in the development of mesopharyngeal cancer and oral cancer.

Research on vocal and swallowing functions

Phonosurgery: We are performing outpatient day surgery using a flexible fiberoptic laryngoscope and performing laryngomicrosurgery using the microflap method under general anesthesia for vocal fold polyps, vocal cord nodules, and vocal cord cysts. For many years we have performed injections of atelocollagen into the vocal folds as outpatient day surgery for unilateral recurrent nerve paralysis; however, we are also performing laryngeal framework surgery for patients who are considered poor candidates for atelocollagen injection.

Diagnosis and treatment of spasmodic dysphonia: Since December 2004 we have performed botulinum toxin treatment as a first-line therapy for spasmodic dysphonia with the approval of the ethics committee of the university. The prevalence of this disorder has been increasing; therefore, evaluating methods for diagnosis and treatment is of clinical importance.

Research on sleep apnea syndrome

To verify whether allergic rhinitis is involved in sleep disorders, research for patients with pollinosis has been performed. Continuous positive airway pressure treatment will be the first choice for patients with obstructive sleep apnea syndrome of greater than moderate severity. On the other hand, the effectiveness and safety of surgical treatment are still unknown. We will be able to present the adaptation of surgical treatment for sleep disorders. Long-distance sleep examinations have been performed since 2009. These research studies are joint projects with the Ota Sleep Science Center.

Publications

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Reviews and Books

Morita Y[1], Tono T[2], Sakagami M[3], Yamamoto Y, Matsuda K[2], Komori M, Hato N[4], Hashimoto S[5], Takahashi H[6], Kojima H ([1]Niigata Univ Graduate Sch Medical and Dental Sciences, [2]Univ Miyazaki, [3]Hyogo Coll Med, [4]Ehime Univ, [5]Natl Sendai Medical Center, [6]Nagasaki Harbor Medical Center). Nationwide survey of congenital cholesteatoma using staging and classification criteria for middle ear cholesteatoma proposed by the Japan Otological Society. *Auris Nasus Larynx.* 2019; **46:** 346-52. Epub 2018 Nov 8.

Department of Anesthesiology

Shoichi Uezono, *Professor and Chairperson*
Naohito Shimoyama, *Professor*
Megumi Shimoyama, *Professor*
Yoshiki Ishiguro, *Professor*
Yasushi Mio, *Professor*
Masanori Takinami, *Associate Professor*
Chieko Fujiwara, *Associate Professor*
Yoichi Kase, *Associate Professor*
Kazuhiro Shoji, *Associate Professor*
Yukako Abukawa, *Assistant Professor*
Kotaro Kida, *Assistant Professor*
Arata Endo, *Assistant Professor*
Kohei Ikeda, *Assistant Professor*
Takuo Yoshida, *Assistant Professor*
Sachiko Omi, *Professor*
Shuya Kiyama, *Professor*
Tsunehisa Tsubokawa, *Professor*
Akihiro Suzuki, *Professor*
Yasuyoshi Sakurai, *Associate Professor*
Ichiro Kondo, *Associate Professor*
Shigehiko Uchino, *Associate Professor*
Hiroshi Sunaga, *Associate Professor*
Gumi Hidano, *Assistant Professor*
Takako Terui, *Assistant Professor*
Toko Fukushima, *Assistant Professor*
Kentaro Yamakawa, *Assistant Professor*
Haściłowicz Tomasz, *Assistant Professor*

General Summary

The functions of the Department of Anesthesiology are to provide quality patient care, to teach, and to perform research in perioperative medicine, intensive care medicine, and comprehensive pain management. In 2018 we made further advances and great achievements with the support of our faculty, institutional administration, and the Dean of The Jikei University. Below we highlight some of our research achievements in 2018.

Research Activities

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

The investigators have been successful each year in obtaining peer-reviewed research grants. In 2018, Grants-in-Aid for Scientific Research were awarded to 5 members of our faculty. The department continues to build on the strengths of several outstanding programs: cardiovascular anesthesia, thoracic anesthesia, pediatric anesthesia, regional anesthesia, neuroanesthesia, obstetric anesthesia, intensive care medicine, and comprehensive pain management.

Our faculty and residents were both well represented at the annual meetings of the Japanese Society of Anesthesiologist in Yokohama and of the American Society of Anesthesiologists in San Francisco. In addition, members of the department continue to be invited as visiting professors or guest speakers at national and international meetings.

Listed below are some of the ongoing research projects in which the principal investigators are faculty members of the Department of Anesthesiology.

Doctors Uezono and Kida have been investigating the protective effects of sedatives in ischemic encephalopathy. Doctor Shimoyama's research has been focused on the mechanism of mitochondria dysfunction-induced peripheral nerve injury. She has also been working to elucidate the mechanism of chemically induced neuropathic pain, which may

lead to new therapeutic interventions.

In clinical medicine, several principal investigators from the Department of Anesthesiology deserve mention. Doctor Kondo has been interested in the postoperative pain treatment service. Doctor Uchino continues to be active in clinical research in the intensive care unit and has been extremely productive in the field of acute kidney injury. As a new faculty member, Dr. Sakurai was recruited to establish an obstetric anesthesia program. He has published many articles related to this field. As an interim chief of the chronic pain clinic, Dr. Uezono has been establishing a new practice of pain management with more invasive procedures, such as spinal cord stimulation. Members of the pain clinic have continued to play a pivotal role in establishing practice guidelines for patients with various types of chronic pain.

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

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Department of Rehabilitation Medicine

Masahiro Abo, *Professor and Chairperson*
Kazushige Kobayashi, *Professor*
Itaru Takehara, *Associate Professor*
Toru Takekawa, *Assistant Professor*
Hidekazu Sugawara, *Assistant Professor*
Kouhei Miyamura, *Assistant Professor*
Shu Watanabe, *Professor*
Nobuyuki Sasaki, *Associate Professor*
Masanori Funakoshi, *Associate Professor*
Kun Suk Chung, *Assistant Professor*
Anri Kamide, *Assistant Professor*

General Summary

The main research topics of our department are: 1) effects of repetitive transcranial magnetic stimulation (rTMS) on brain injury, 2) treatment for stroke, and 3) analysis based on database.

Research Activities

Effects of rTMS

1. We studied the relationship between sleep during low-frequency rTMS session and motor function improvement in upper limb in post-stroke patients. The bispectral index (BIS) monitor was used to investigate the relationship. The patients were sorted into awake and asleep groups based on the BIS change during rTMS sessions. Action Research Arm Test was significantly improved in asleep group compared to awake group, while Fugl-Meyer assessment was not. Sleep during low-frequency rTMS may be associated with motor improvement in upper limb hemiparesis.
2. At early stage for the upper limb paresis, healthy brain hemisphere is not yet overactive and high frequency rTMS to the damaged side is effective. For lower limbs, it is better to apply during chronic phase. Frontal lobe rTMS for apathy may also be beneficial.
3. We have been applying rTMS for aphasia since 2009 along with the therapy. Preconditioning, interhemispheric inhibition, and usage of fMRI are explained as the basis of our treatment.

Treatment for stroke

1. Motor function and spasticity

1) The effects of repetitive botulinum toxin A therapy (BoNT-A) and intensive rehabilitation (IR) on lower limb spasticity in post-stroke patients were retrospectively studied. The treatment improved lower limb spasticity in post-stroke patients, and those who show the forward gait pattern prior to therapy tended to be freed from the braces after therapy.
2) We retrospectively investigated the relationship between BoNT-A combined with multidisciplinary rehabilitation and muscle echo intensity in post-stroke patients with spasticity. We observed significant improvements in the modified Ashworth scale scores after the treatment, especially in those with lower echo intensity.

2. Higher brain dysfunction

1) We discussed five current issues on higher brain dysfunction: lack of social understanding, incomplete rehabilitation system, difficulty in differentiating social and psychological issues, diagnostic challenge, and needs for social support for return-to-work.
2) The approach to return-to-drive for post-stroke patients was discussed based on multipronged analysis.
3) The questionnaire was performed to patients' family, regarding the disease, home environment, and social support.

Analysis based on database

1. Impact of orthotic therapy for improving motor ability in activities of daily living in individuals with spinal cord injury was discussed as a retrospective cohort study. Leg orthoses may improve activities of daily living in individuals with spinal cord injury after the acute phase.

Publications

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Department of Emergency Medicine

Satoshi Takeda, *Professor*
Kei Ohtani, *Associate Professor*
Taro Nameki, *Assistant Professor*
Masahiko Uzura, *Professor*
Kenji Okuno, *Associate Professor*
Takeki Ogawa, *Professor Emeritus*

General Summary

1. Education system for junior residents in emergency medicine
2. Establishing a database of severe traumatic brain injuries in Japan
3. The etiology of syncope
4. Research on laboratory assessment of myocardial infarction in the emergency room
5. Managing a course on immediate cardiac life support (ICLS)
6. Managing the Japan Advanced Trauma Evaluation and Care (JATEC) course
7. Providing logistical support to the Japan Boxing Commission
8. Basic research on traumatic brain injury
9. Basic and clinical research on oxidative stress and emergency medicine
10. Advice to local authorities on plans for disaster medicine
11. Creation of a Disaster Medical Assistance Team (DMAT) deployment system
12. Management of a hospital emergency response drill, including "code blue" ("stat call") and the rapid response system (RRS)
13. Managing the Jikei Airway Management course for patient safety (JAMP) course
14. Providing logical support for the Japan AED Foundation

Research Activities

1. Supervision and development of ultrasound devices for the diagnosis and treatment of cerebrovascular disorders
2. Director of the Japan Neurotrauma Data Bank Committee
3. Prognostic value of heart fatty acid-binding protein for patients with chest symptoms in the emergency room
4. Research committee on higher cerebral function after traumatic brain injury
5. Research committee on impact biomechanics in automobile accidents (Society of Automotive Engineers of Japan
6. Published a revised edition of *Guidelines for the Treatment and Management of Severe Head Injury* (The Japan Society of Neurotraumatology)
7. Research group on cerebrospinal fluid in cases of traumatic intracranial hypotension
8. Basic research on traumatic brain injury and oxidative stress
9. Basic research on heat stroke and neuronal injury
10. Development of anti-free-radical therapy in patients with acute neuronal conditions
11. Development of educational system in emergency medicine, including the use of simulation training

Publications

Mitsunaga T, Ohtaki Y, Kiriyama N, Ohtani K, Yajima W, Hibi T, Takeda S. Characteristics of patients hospitalised in an emergency department observation unit in Japan. *ECJ* [Internet]. 2 Aug 2018 [cited 26 Aug 2019]; **14**. Available from: https://www.pagepressjournals.org/index.php/ecj/article/view/7381

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Department of Laboratory Medicine

Tomokazu Matsuura, *Professor*
Hiroshi Yoshida, *Professor*
Kenichi Sugimoto, *Professor*
Yoji Ogasawara, *Associate Professor*
Yoshihiro Mezaki, *Assistant Professor*
Midori Kouno, *Assistant Professor*
Ken Kaito, *Professor*
Hironari Sue, *Professor*
Kouji Nakada, *Professor*
Takahiro Masaki, *Assistant Professor*
Sae Ochi, *Assistant Professor*
Setuko Akizuki, *Assistant Professor*

General Summary

We performed a wide range of research in clinical laboratory medicine, including practical studies of infectious disease tests, biochemical tests, blood tests, physiological function tests, and clinicopathological tests. In addition, future subjects of our department will be of the development of collection methods of clinical information, medical safety measures, the new development of brain function tests, application to clinical tests of mass spectrometry, and functional tests using stable isotope ^{13}C-labeled compounds.

Research Activities

Clinical microbiology

We participated in medical education programs to provide basic clinical skills training for medical students. In addition, we were appointed to be examiners of objective structured clinical examinations for students in years 4 and 6. Basic and clinical research on viral hepatitis and liver fibrosis was conducted, and our manuscript about transforming growth factor β and liver fibrosis was published in the journal *Heliyon*. We also investigated the effects of metoformin, a widely used pharmacotherapeutic agent for type 2 diabetes, on intestinal microflora in mice. We attempted to perform the chromatographic analysis of volatile organic compound patterns in exhaled breath from patients with inflammatory diseases.

Clinical chemistry

We studied gastric emptying and fat digestive and absorptive function after various types of gastrectomy by ^{13}C-breath tests. Function-preserving gastrectomy attenuated rapid gastric emptying, which is usually seen after conventional gastrectomy. This attenuation might, in part, explain the mechanism of ameliorating postgastrectomy syndromes, such as diarrhea and dumping.

Professor Yoshida and others demonstrated that the diet therapy of the weight-loss program decreased chylomicron cholesterol and decreased cholesterol/alpha tocopherol of lipoprotein(a) (unpublished data). In the meantime, we have reported that angiotensin II can enhance matrix metalloproteinase 2, mainly through angiotensin II receptor type 2, in endothelial cells (J Cardiovasc Pharmacology 2018; 71: 233-9).

Safety management in clinical laboratories

Because accidents occurring in a laboratory sometimes have harmful results, software and hardware must be improved and safety measures, based on an understanding of human thought, must be ensured. We analyzed situation monitoring, situation awareness, and action to complete the safer work. When accidents occurred in our department, they were due to inadequate observation and confirmation, 80%; communication errors, 11%; and poor procedures, 9%. Most of the accidents were derived from heuristics brought by quick thought after recognition of the target. To avoid the judgment by heuristics, basic confirmation practices should be customarily performed.

Clinical hematology

We are studying the pathophysiology of bone marrow failure syndrome. To assess the optimal treatment for acquired aplastic anemia we analyzed the clinical and laboratory data of patients with aplastic anemia who received immunosuppressive therapy at our hospital.

Clinical psychiatry

We discussed a patient with nonconvulsive status epilepticus and psychic problems. We have conducted a study of epilepsy in adult patients taking Resilience into consideration and a study of the reconsideration of rational antiepileptic drug polypharmacy. We held the 12th Kanto-Koushinetsu regional epilepsy meeting in June. We are planning to establish the Epilepsy Center of The Jikei University Hospital.

Clinical physiology

Research was conducted on the accuracy of automatic electrocardiogram analysis for diagnosing arrhythmia. In the clinical area, we continued research related to catheter ablation of atrial fibrillation and reported the results in several journals.

Clinical pathology and clinical cell biology

Vitamin A is needed for many physiological functions, including vision, reproduction, embryonic development, and maintenance of epithelia. We have analyzed cellular retinol-binding protein I and lecithin:retinol acyltransferase in polar bear liver, which contained a large amount of vitamin A, and found a strong co-localization of these 2 proteins.

Publications

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Reviews and Books

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Department of Endoscopy

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Keiichi Ikeda, *Associate Professor*
Hirobumi Toyoizumi, *Assistant Professor*
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General Summary

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

Research Activities

Pharyngeal, esophageal, gastric, duodenal, and colonic malignancies

1. Endoscopic diagnosis of neoplasia in the GI tract

Early detection and accurate diagnosis of premalignant and malignant lesions in the GI tract are essential for selecting the most appropriate therapeutic strategy for each patient. To evaluate these clinical cases we use several novel optical technologies, along with conventional white-light endoscopy. We have designed a series of prospective clinical studies to evaluate and validate these novel imaging technologies and their potential benefits.

a) Magnifying endoscopic observation with an NBI system

This new diagnostic system consists of a magnifying (× 90) endoscope and an NBI light source and provides detailed morphological information about the capillaries on the mucosal surface of neoplastic lesions. We are investigating the clinical utility of NBI-magnifying endoscopy for identifying superficial neoplasms and developing algorithms that would allow the histological type and tumor extent of GI tract neoplasia to be determined. On the basis of our findings with magnified NBI, we have developed a novel classification system for gastric cancer and demonstrated, in a prospective study, its advantages over the conventional diagnostic system. We also joined a multicenter study of NBI-magnifying endoscopy for detecting superficial carcinomas of the pharynx and esophagus. Accurate preoperative evaluation of the depth of invasion into the submucosal layer is essential for appropriate decision-making and for determining the optimal therapeutic strategy for patients with colonic lesions. To maximize our diagnostic accuracy, we use this magnifying endoscope with NBI and crystal-violet staining. Results of these studies have been reported at several conferences and have been published in several English-language journals.

b) Confocal laser endomicroscopy

We introduced confocal laser endomicroscopy, which provides subsurface imaging of the

GI walls with image quality equivalent to that of bench confocal microscopy. We have joined an international multicenter study to assess the advantages of confocal laser endomicroscopy over conventional endoscopy for differentiating gastric neoplasia from non-neoplastic mucosa. The results were reported at international meetings and published in an internationally renowned scientific journal.

2. Endoscopic treatment of esophageal, gastric, and colonic malignancies

Recent advances in endoscopic diagnostic techniques and instrumentation have led to the expansion of indications for endoscopic therapy in GI tract carcinomas. We now perform endoscopic submucosal dissection (ESD) for superficial neoplasms of the esophagus, stomach, and colon. En bloc resection with ESD is considered necessary to further develop endoscopic treatment. Successful development of a series of endoscopic knives traction devices and submucosal injection fluids reduced the technical difficulty of ESD and the risk of complications.

3. Diagnosis and treatment of oropharyngeal and hypopharyngeal malignancies

Detecting cancer at an early stage is important. We have found that, in combination with the NBI system, magnifying endoscopy has allowed hard-to-find cancers to be detected at an early stage, without the need for Lugol chromoendoscopy. In collaboration with the department of otorhinolaryngology, we introduced endoscopic removal of early-stage cancers in this area and are evaluating clinical outcomes.

Enteroscopy

1. Diagnostic techniques

Capsule endoscopy is a breakthrough examination that can be used to detect lesions in parts of the small intestine that are inaccessible with an ordinary endoscope system. However, because capsule endoscopy is purely diagnostic, we have introduced single-balloon enteroscopy, which allows biopsy and hemostasis to be performed for hemorrhagic lesions of the small intestine.

Pancreatobiliary endoscopy

1. Diagnosis of biliary and pancreatic diseases

The establishment of a standardized, systematic diagnostic algorithm for biliary and pancreatic diseases are extremely important. We are comparing the diagnostic accuracy for hepatopancreatic diseases of endoscopic ultrasound-guided fine needle aspiration biopsy, multidetector-row computed tomography, magnetic resonance cholangiopancreatography, and endoscopic retrograde cholangiopancreatography. We are developing new diagnostic markers for pancreatic carcinoma and a system for their measurement. We will be applying these markers to the differential and prognostic diagnosis of pancreatic carcinoma with specimens obtained with endoscopic ultrasound-guided fine needle aspiration biopsy.

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Department of Infection Control

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Hiroki Tsukada, *Professor*
Tetsuya Horino, *Associate Professor*
Koji Yoshikawa, *Assistant Professor*
Masaki Yoshida, *Professor*
Yasushi Nakazawa, *Associate Professor*
Hiroshi Takeda, *Assistant Professor*

General Summary

We investigate infectious diseases at the points of the host, pathogen, antimicrobial agents, and infection control. This year we investigated patients with syphilis and with *Staphylococcus aureus* bacteremia, Yezo sika deer as a reservoir of *Toxoplasma*, and the efficacy of carbapenem for patients with severe acute pancreatitis.

Research Activities

Clinical analysis of trends and diagnosis among patients with syphilis
We retrospectively performed clinical analysis of 64 patients with active syphilis. Symptomatic syphilis was diagnosed in 43 patients. Diagnosing syphilis had been difficult in 4 patients because other diseases had been suspected. Early symptomatic syphilis was diagnosed in 7 patients on the basis of clinical symptoms and positive results of a treponemal, low nontreponemal test. Asymptomatic syphilis was diagnosed in 21 patients. Syphilis was diagnosed in many patients by means of screening for infectious disease. In 2 patients the diagnosis was delayed owing to the oversight of qualitative test results. To diagnose syphilis, clinicians must make a comprehensive judgement on the basis of test results and clinical findings.

Seroprevalence of Toxoplasma gondii *in wild sika deer in Japan*
Toxoplasmosis is a food-borne infection that is widespread around the world, causing congenital disorders and opportunistic infections. Ingestion of undercooked meat is a risk factor for infection with the causative agent, *T. gondii*. Japanese people occasionally eat rare meat as a traditional cuisine style called *sashimi*. The consumption of venison has rapidly increased in Japan, mainly because of the enhanced population control of wild Japanese sika deer (*Cervus nippon*). In particular, the meat of Yezo sika deer (*C. nippon yesoensis*) in Hokkaido (the northernmost and largest prefecture of Japan) is frequently supplied to markets as branded game/bushmeat. To study the possible burden of *T. gondii* among wild Yezo sika deer, plasma samples of deer hunted during 2 seasons, 2010 to 2012, in Eastern Hokkaido were investigated. A total of 80 samples were examined with the Sabin-Feldman dye test, which is highly specific and sensitive for identifying the development and persistence of antibodies after primary *Toxoplasma* infection; 38 cases (47.5%) were seropositive (cut-off titer < 1:16). The antibody prevalence of *T. gondii* was higher in does than in bucks. The seroprevalence was higher in adult deer 3 years or older than in younger animals. The overall seroprevalence fluctuated significantly according to the season when the deer were hunted. These results indicate widespread infection with *T.*

gondii among Japanese wild Yezo sika deer and suggest that both appropriate handling and treatment of bushmeat are required to prevent food‐borne toxoplasmosis in Japan.

Early prophylactic antibiotics for severe acute pancreatitis: a population‐based cohort study of a nationwide database in Japan
Previous studies evaluating the clinical benefits of prophylactic antibiotics for severe acute pancreatitis have had inconsistent results owing to heterogeneities among the study settings. To determine whether early prophylactic antibiotics improve the outcomes of patients with severe acute pancreatitis, we performed a study designed to overcome these previous methodological weaknesses. With a Japanese nationwide inpatient database we conducted a retrospective cohort study of patients with severe acute pancreatitis discharged from July 2010 through March 2016. We divided patients into those with early prophylactic carbapenem use (prophylaxis group) and those without prophylaxis (control group). The primary outcome was in‐hospital mortality, and the secondary outcomes included oral vancomycin use. We identified a total of 3,354 eligible patients, including 2,493 in the prophylaxis group and 861 in the control group. The overall in‐hospital mortality rate was 12.8%. Prophylactic antibiotics were not significantly associated with a reduced rate of in‐hospital mortality according to Cox regression analysis (hazard ratio [HR], 0.88; 95% confidence interval [CI], 0.62‐1.23) or instrumental variable analysis (risk difference, 1.2%; 95% CI, 9.8%‐7.4%). However, prophylactic antibiotic use was significantly associated with in‐hospital oral vancomycin use in a competing‐risk model (subdistribution HR, 1.91; 95% CI, 1.02‐3.56).
Conclusions: The present study suggests that routine early prophylactic antibiotic use has no significant clinical benefit in patients with severe acute pancreatitis but might increase the risk of hospital‐acquired infections.

Predictive factors for metastatic infection in patients with bacteremia caused by S. aureus
To determine the predictive factors for metastatic infections with *S. aureus* bacteremia, we conducted a retrospective cohort study among patients with *S. aureus* bacteremia in The Jikei University Kashiwa Hospital. Enrolled in the study were 74 adult patients with *S. aureus* bacteremia hospitalized from January 2014 through December 2017. The most common primary site of bacteremia was catheter‐related (24 [32.4%] of 74 patients). Metastatic infection occurred in 22 (29.7%) of 74 patients, and spondylitis was most common, following a psoas abscess. Of these 22 infections, 11 (50%) were community acquired. No significant differences were found in demographics and comorbidities, except central venous catheter‐associated bloodstream infection, which was associated with low rate of metastatic infection. Multivariate analysis showed that the predictive factors associated with the development of metastatic infection were community onset of infection (odds ratio [OR], 11.6; 95% CI, 2.98‐45.1; $P < 0.001$), fever for more than 72 hours (OR, 6.7; 95% CI, 2.12‐21.8; $P = 0.001$), and higher C‐reactive protein levels (> 3 mg/dl) lasting 2 weeks after the administration of appropriate antibiotics (OR, 7.47; 95% CI, 2.39‐23.3; $P < 0.001$). Therefore, additional diagnostic tests to identify metastatic infection should be performed, especially in patients with community‐acquired *S. aureus* bacteremia, persistent fever, or persistently high C‐reactive protein levels after the admin-

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Department of Dentistry

Katsuhiko Hayashi, *Professor*
Shigeru Suzuki, *Associate Professor*
Akihiro Ikai, *Professor*

General Summary

1. Changes in condylar heights in the temporomandibular joint with anterior disc displacement without reduction using panoramic radiographs
2. Tumor necrosis factor (TNF) α receptor (R)–P2X3R mechanisms in trigeminal ganglion neurons are involved in orofacial neuralgia associated with macrophage infiltration in a rat model of trigeminal nerve compression (TNC)
3. CD57 immunoreactivity seen in thin arteries in the human fetal lung
4. Morphological study of the fibula in Japanese: Basic anatomical study for maxillofacial reconstruction

Research Activities

Changes in condylar heights in the temporomandibular joint with anterior disc displacement without reduction using panoramic radiographs

The aim of the study was to measure condylar heights and to quantitatively assess the changes in condylar heights in patients with anterior disc displacement without reduction by measuring condylar heights on panoramic radiographs. The subjects of this study were selected from among patients who had no anterior disc displacement or had anterior disc displacement with or without reduction. These diagnoses were confirmed with magnetic resonance imaging. We compared, via quantitative and statistical examination, differences of right and left condylar heights among these 3 groups. In the groups with anterior disc displacement with or without reduction, condylar heights were significantly lower on the affected side than on the unaffected side. However, age, condylar height, and the difference between affected and unaffected sides did not differ significantly between these 2 groups. In the group with anterior disc displacement without reduction, we used receiver operating characteristic curves to compared condylar heights of affected sides and of unaffected sides and how condylar heights differed between the sides. According to the study, the most appropriate way for estimates of the changes in condylar heights in patients with anterior disc displacement without reduction is to assess differences between both condylar heights.

TNFαR–P2X3R mechanisms in trigeminal ganglion neurons are involved in orofacial neuralgia associated with macrophage infiltration in a rat model of TNC

We developed a model of trigeminal nerve compression by placing a glass rod on the trigeminal root of male Sprague–Dawley rats (180–200 g). We then studied the expression of TNFα, TNFαR, and P2X3R and macrophage infiltration immunohistochemically in the trigeminal ganglion and also studied the head-withdrawal threshold (HWT) to mechani-

cal stimulation of the whisker pad skin. After decompression of the trigeminal nerve, the mechanical HWT was significantly lower in TNC rats than in sham rats. The numbers of P2X3R-immunoreactive (IR) and TNFαR-IR neurons innervating whisker pad skin and of neurons encircled with cells positive for ionized calcium-binding adapter molecule 1 (Iba1) were significantly higher in TNC rats than in sham rats. In addition, TNFα was expressed in iba1-IR cells. Furthermore, the decreased HWT was significantly recovered following local injection of a P2X3R antagonist. Trigeminal nerve decompression might be a reliable model of trigeminal neuralgia, and the present results suggest that TNFαR-P2X3R signaling associated with TNFα release from activated macrophages is involved in trigeminal neuralgia.

CD57 immunoreactivity seen in thin arteries in the human fetal lung

In lung specimens from 12 human fetuses at 10 to 34 weeks of gestation, we have incidentally found that segmental, subsegmental, and more-peripheral arteries strongly expressed CD57. The CD57-positive tissue elements within intrapulmonary arteries seemed to be the endothelium, internal elastic lamina, and smooth muscle layer, which corresponded to tissue positive for an antibody reactive with smooth muscle actin. However, the lobar artery, pulmonary arterial trunk, and bronchial arteries were negative for CD57. Likewise, arteries in and along any abdominal viscera, such as the heart, thymus, and thyroid, did not express CD57. Thus, the lung-specific CD57 reactivity was not connected with an endodermal origin or a branchial arch origin. The CD57 antigen is a sugar chain characterized by a sulfated glucuronic acid residue that is likely to exist in some glycosphingolipids. Therefore, a chemical affinity or an interaction might exist between CD57-positive arterioles and glycosphingolipids originating from alveoli, resulting in acceleration of capillary budding to make contact with the alveolar wall. We conclude that CD57 might therefore be a functional marker of the developing air-blood interface that characterizes the fetal lung at the canalicular stage.

Morphological study of the fibula in Japanese: Basic anatomical study for maxillofacial reconstruction

In this study, the 3-dimensional morphology of the fibula of Japanese patients was observed, the previously unreported distribution and location of the feeding blood vessels were examined, and the region containing sufficient bone mass for maxillofacial reconstruction was anatomically evaluated. Images of the excised fibula were acquired with computed tomography for medical use and subjected to 3-dimensional reconstruction. Before being measured, the fibula was divided into 6 parts between the apex of the fibular head and the apex of the lateral malleolus. The 4 central regions were set as regions of interest, and the diameters of the fibular cross sections were 3-dimensionally measured. In addition, the distribution of the feeding blood vessels to the fibula was macroscopically observed and classified. On 3-dimensional bone morphology measurement, the width (diameter) of the fibula cross section between the anterior margin and the posterior aspect was the greatest, followed by that between the medial crest and the lateral aspect. In the regions of interest, the mean width between the anterior margin and the posterior aspect, which corresponded to the grafted bone height, exceeded 10 mm, and the mean width

between the medial crest and the lateral aspect and between the posterior margin and the medial aspect, which became the buccolingual width of the grafted bone, exceeded 6 mm, confirming that the graft thickness is sufficient for grafting. Furthermore, the blood vessels feeding the fibula entered the bone in the central one-third region in all preparations. Inclusion of the central one-third region was suggested to be effective for vascularized fibular grafting.

Publications

Ishizuka S*[1]*, Jin Z-W*[2]*, Yamamoto M*[1]*, Murakami G*[1]*, Takayama T, Hayashi K, Abe S*[1] *([1]Tokyo Dental College, [2]Jiangnan Univ). CD57 (Leu-7, HNK-1) immunoreactivity seen in thin arteries in the human fetal lung. *Anat Cell Biol.* 2018; **51:** 105-12.

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Department of Transfusion Medicine and Cell Therapy

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General Summary

Patient blood management is an evidence-based approach to optimize the care of patients who might need blood transfusions, as described by the American Association of Blood Banks. The term "patient blood management" was first used in 2005 by Prof. James Isbister, an Australian hematologist, to emphasize the importance of improving the outcomes of patients after transfusion. In other words, the focus during transfusion should be changed from blood products to patient care. To achieve this goal, the cooperation of nurses is essential and has been enhanced by the introduction of transfusion nursing certification by the College of Transfusion Nurses in Japan in 2010 by Prof. Tasaki, Prof. Hitoshi Ohto (Fukushima Medical University) and others. Therefore, we teach nurses inside and outside of The Jikei University Hospital about their role in transfusion medicine, including the appropriate and safe use of blood products.
The main research activities in 2018 were as follows: (1) the efficacy of cryoprecipitates in patients with massive blood loss during surgery, (2) the risk of bacterial contamination of blood for transfusion when connecting the transfusion set to the blood bag, (3) the significance of leukoreduction of autologous blood, (4) the production of alloantibodies in children, (5) the effectiveness of HLA-matched platelets in patients with platelet transfusion refractoriness, and (6) a report on a rare case of delayed hemolytic transfusion reaction due to anti-Kell antigen antibodies.

Research Activities

Efficacy of cryoprecipitates in patients with massive blood loss during surgery
In patients with massive blood loss, hemostasis can be difficult owing to the consumption and dilution of coagulation factors. In Japan, however, no commercially available cryoprecipitates are available for such patients, and fresh frozen plasma (FFP) is often used. Therefore, in January 2017 we began preparing cryoprecipitates derived from allogeneic FFP for patients undergoing cardiac surgery, during which massive blood loss and a need for FFP are possible. In a pilot study of 14 patients in whom cryoprecipitate was used, significant hemostasis was observed. In the next stage, overall evaluation based on such variables as total units of blood used, operation duration, length of hospitalization, and cost-effectiveness will be needed to show the significance of cryoprecipitate use for the treatment of massive blood loss.

Bacterial contamination of blood when a blood bag and a transfusion set are connected
Bacterial contamination of transfused blood can severely endanger patients. Although blood is drawn and stored in a blood center, it is not always properly handled at the bedside. When a blood bag and a transfusion set are connected, blood rarely leaks but can

leak via blood bag breakage or a loose connection. We have experimentally examined how blood is contaminated with bacteria, for example, if a transfusion set is prepared with unclean, ungloved hands.

Significance of leukoreduction of autologous blood
Prestorage leukoreduction of blood components is often performed to avoid adverse events. However, whether this procedure is beneficial for autologous blood is unknown. To identify possible benefits, we performed a prospective randomized crossover study in which patients undergoing 2-stage bilateral total hip arthroplasty received either leukoreduced blood (93 patients) or non-leukoreduced blood (99 patients) during the first operation and received the other type of blood during the second operation. The results of this study did not show any superiority of prestorage leukoreduced autologous blood over non-leukoreduced autologous blood.

Production of alloantibodies in children
Whether neonates are able to produce alloantibodies against red blood cell alloantigens is unclear. Professor Yoshiko Tamai (Hirosaki University) and colleagues started the distribution of questionnaires regarding this issue to hospitals where more than 30 children per year receive allogeneic blood.

Effectiveness of HLA-matched platelets in patients with platelet transfusion refractoriness
Platelet transfusion refractoriness due to alloantibodies to platelets is a serious problem in patients frequently needing platelet transfusion. In these cases, transfusion of HLA-matched platelets seems effective. However, the use of cross-matched positive platelets is unavoidable in an emergency situation. Hagino et al. evaluated the effectiveness of such platelets on the basis of the 1-hour posttransfusion corrected count increment.

A case of delayed hemolytic transfusion reaction due to anti-Kell antigen antibodies
Hemolytic transfusion reactions due to antibodies against low-incidence red blood cell Kell antigens (Kp^c) have rarely been reported in Japan or in other countries. We report a rare case of hemolysis after a homologous blood transfusion which was subsequently shown to be caused by anti-Kp^c antibodies.

Publications

Ikeda K*[1]*, Ohto H*[1]*, Okuyama Y*[2]*, Fujiwara M*[3]*, Kanamori H*[4]*, Fujiwara S*[5]*, Muroi K*[5]*, Mori T*[6]*, Kasama K, Iseki T*[7]*, Nagamura-Inoue T*[8]*, Fujii N*[9]*, Ashida T*[10]*, Kameda K*[5]*, Kanda J*[11]*, Hirose A*[12]*, Takahashi T*[13]*, Nagai K*[14]*, Minakawa K*[1]*, Tanosaki R*[15] *([1]Fukushima Medical Univ, [2]Komagome Hosp, [3]Tohoku Univ, [4]Kanagawa Cancer Center, [5]Jichi Med Univ, [6]Keio Univ, [7]Chiba Univ, [8]Tokyo Univ, [9]Okayama Univ, [10]Kindai Univ, [11]Saitama Med Center, [12]Osaka City Univ, [13]Shimane Univ, [14]Nagasaki Univ, [15]National Cancer Center). Adverse Events Associated With Infusion of Hematopoietic Stem Cell Products: A Prospective and Multicenter Surveillance Study. *Transfus Med Rev.* 2018 Jun 1. pii: S0887-7963(18)30023-3.

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Department of Molecular Physiology
Division of Physical Fitness

Shigeru Takemori, *Professor and Director* Hideki Yamauchi, *Assistant Professor*

General Summary

Research activities in our division have been focused on the plasticity of skeletal muscle and preventive medicine against sarcopenia and metabolic syndrome in terms of exercise physiology.

Research Activities

Dysfunction of autophagy in old rats and unloading-induced sarcopenia

Unloading is known to induce sarcopenia in old rats with histological features characteristic of autophagy dysfunction. We have reported that intermittent resistance-exercise (IRE) ameliorates this sarcopenia. Here, we aimed to clarify the effects of IRE on autophagy in unloaded muscles of old rats. Old female F344 rats (2 years old, n = 21) were randomly divided into control, unloading, and unloading + IRE groups. Middle-aged rats (1 year old, n = 7) were used as an adult control group. Rats of the unloading and unloading + IRE groups had their hindlimbs unloaded by tail suspension. In the unloading + IRE group, IRE was performed 3 times per day for 10 minutes at 4-hour intervals in a dark period. The intervention period was 3 weeks. The mass of the lateral gastrocnemius was significantly lower in old control rats than in adult control rats. On the other hand, unloading-induced atrophy was more prominently observed in the soleus. The IRE ameliorated atrophy in both the lateral gastrocnemius and the soleus. In the red part of type I-rich lateral gastrocnemius, unloading selectively affected type I fibers disrupting myofibrils with a decrease in sarcomeric proteins, formation of the inclusion body, and accumulation of abnormal mitochondria. The IRE inhibited such type I fiber-specific disruptions. In type I-rich soleus, a significant decrease in myofibrilar protein was found to accompany increases in ubiquitinated proteins and ubiquitin ligase (Fbx32) and decreases in microtubule-associated protein light chain 3 (LC3)-II and mitochondrial calcium uniporter (MCU). The IRE increased myofibrillar protein, LC3-II, parkin, and MCU in the soleus. We conclude that functional alteration in autophagy and mitophagy might be involved in the effects of unloading and IRE prominently in type I fibers.

Increased muscle stiffness after eccentric contraction

We explored the cause of an increase in muscle stiffness after eccentric contraction (ECC) in rat plantaris muscle. During 0.3-second-long tetanic ECC elicited through tibial nerve stimulation in anesthetized 8-week-old male F344 rats, the plantaris muscle was stretched to the length for maximal isometric twitch tension (L0) from 0.9 L0. The H10, M10, and L10 groups received 10 ECCs elicited by 100, 75, and 50 Hz supramaximal stimulation, respectively. The L30 group received 30 ECC by 50 Hz stimulation. The ISO group

received submaximal isometric tetanus contraction by 100 Hz stimulation, and the CON group received no conditioning contraction. After the series of contractions, plantaris muscle was dissected for analysis with x-ray diffraction, electrophoresis, and the measurement of myofibrillar resting tension. The ECC in the H10 group significantly increased muscle stiffness. The deterioration of the microstructural sarcomere detected with x-ray diffraction patterns was slight in the ISO, L10, and L30 groups but was moderate in the M10 group and marked in the H10 group. The profiles of α-connectin, β-connectin, and myofibrillar resting tension did not differ significantly between the H10 group and the ISO group. On the basis of these results, we conclude that the increase in muscle stiffness in the H10 group is not due to any change in connectin. The increase in muscle stiffness might, therefore, represent a slight increase in the concentration of intracellular Ca^{2+} due to microscopic cellular injuries.

An x-ray diffraction study on in vivo skeletal muscle with maintained blood supply

X-ray diffraction is a fixation-free technique that enables the function and structure of living skeletal muscle to be evaluated. Its application to in vivo muscles with a maintained blood supply is expected to be of high potential in physiological studies of muscle adaptations, such as atrophy and hypertrophy. Each periodic repeat in sarcomeres gives rise to a specific series of reflections or layer-lines on an x-ray diffraction pattern. The intense reflection pairs (1,0 and 1,1 reflections) eminated bilaterally represent myofilament lattice reflecting volume change and the vital state of cells. As an initial attempt to apply this technique to the evaluation of in vivo muscle, we examined muscle states at very early days after denervation. A unilateral incision was made on the sciatic nerve of 6-month-old female ICR mice, with a sham operation on the other side. X-ray diffraction patterns from the extensor digitorum longus muscle of anesthetized mice were obtained 0, 0.5, 2, and 5 days after the operation at the BL-6A beamline for small angle X-ray scattering at the High Energy Accelerator Research Organization, Tsukuba. The patterns showing 1,0 and 1,1 reflections and several layer-lines representing helical arrangement of myoproteins were successfully obtained. Although muscles showed no signs of atrophy in tissue wet weight or the connectin/titin profile, 1,0 and 1,1 reflections of denerved muscles suggested cellular swelling in 0.5 day and subsequent partial deprivation of ATP in 5 days. We succeeded in obtaining x-ray diffraction patterns from in vivo muscle with a maintained blood supply. The pattern indicates very early effects of denervation on muscle cells.

Effect of exercise on the accumulation of ingested polyamine in various tissues of rats

Polyamines, such as putrescine, are polycation molecules indispensable for cell proliferation. Polyamines are also reported to modulate cardiac excitability and cell viability. We have recently found that exercise suppressed accumulation of ingested putrescine in the heart and serum of rats. In the present study, we examined the effects of exercise on the accumulation of ingested putrescine in other tissues. Six-week-old female Wistar rats were fed with drinking water containing 1 mg/ml putrescine for 9 weeks with or without a freely accessible wheel for spontaneous running. Their skeletal muscle, liver, lung, spleen, and fat were then dissected for polyamine content analysis with high-performance

liquid chromatography. Urine adsorbed on filter papers covering the cage bottom was also analyzed for polyamine metabolites. Exercise significantly suppressed the accumulation of ingested putrescine in all tissues tested. Although both putrescine ingestion and exercise induced no significant difference in tissue spermidine content, urinary spermidine showed a significant increase. Because spermidine is a metabolite of putrescine, we speculate that the ingested putrescine might be rapidly catabolized. With the possibility that exercise affects polyamine metabolism, more comprehensive analysis is required of metabolites with stable-isotope tracer analysis by mass spectrometry.

Department of Cell Physiology
Division of Aerospace Medicine

Susumu Minamisawa, *Professor*

General Summary

Our main research interests are gravitational physiology and aerospace medicine.

Research Activities

Regulation of cytosolic Ca^{2+} concentration in Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD), the most common and severe form of muscular dystrophy in childhood, is an X-linked disease caused by deficiency of dystrophin protein in muscle. The absence of dystrophin causes increased Ca^{2+} influx and an abnormal cytosolic Ca^{2+} homeostasis of myofibers, leading to increased necrosis. Several underlying mechanisms have been suggested to cause this abnormal cytosolic Ca^{2+} regulation in dystrophic muscle. Therefore, the aim of this study was to identify new therapeutic targets based on cytosolic Ca^{2+} regulation for DMD. Among several mechanisms, a decrease in the activity of sarco(endo)plasmic reticulum calcium ATPase (SERCA) has been considered as a primary cause of cytosolic Ca^{2+} overload in DMD, because SERCA accounts for > 70% of Ca^{2+} removal from cytosol. We found that the level of sarcolipin, an intrinsic inhibitory sarcoplasmic reticulum protein of SERCA, is abnormally high in dystrophic muscle. In this study, we determined the physiological relevance of sarcolipin in DMD following loss-of function by deletion of the gene sarcolipin (*SLN*). Knockdown of *SLN* ameliorated the cytosolic Ca^{2+} homeostasis and the dystrophic phenotype in a mouse model of DMD. These findings suggest that *SLN* is a novel target for DMD therapy.

In addition, we found a significant decrease in the muscle weight and cross sectional area of muscle fibers, although the number of fibers did not differ with the increased cytosolic Ca^{2+} concentration. For the mechanisms of reduction in the cross-sectional area and weight, we considered 3 possibilities, namely fiber atrophy, fiber type changes, and autophagy. An increasing number of type 2A fibers and the atrophy of type 2B fibers were observed. Peroxisome proliferator-activated receptorγ coactivator-1α (PGC1α), which can alter fiber types from fast to slow, was up-regulated in the tibialis anterior muscle. Also up-regulated were atrogin-1, muscle RING finger protein 1, and phosphorylation of Forkhead Box class O, which can induce muscular atrophy. In contrast, autophagy signals, such as microtubule-associated protein 1 light chain 3 (LC3) A and B, were not altered. These results suggest that an increase in cytosolic Ca^{2+} concentration changes the muscle fiber type and induces muscle atrophy.

Phenotypic analysis of juvenile onset dilated cardiomyopathy mouse model

Dilated cardiomyopathy (DCM) is characterized by cardiac dilation and pump failure. A fundamental therapy for DCM has not been established. In particular, DCM that develops

at a young age has a poor prognosis. The troponin T amino acid mutation (ΔK210) knock-in mouse (ΔK210-KI), generated by Dr. Sachio Morimoto and others, is considered to have the similar phenotype as a human child with juvenile DCM (Circ Res. 2007; 101: 185-94). However, neither the neonatal period nor the weaning period has been examined in detail. The purpose of this study was to investigate cardiac pathology and changes in gene expression in ΔK210-KI during the neonatal and weaning periods and to identify early progression factors of DCM. We found that cardiac hypertrophy has already developed at birth in homozygous ΔK210-KI mice. Furthermore, we are doing research to develop gene therapy by replacing mutant troponin T with normal troponin T overexpression.

Molecular mechanisms of intracellular Ca^{2+} mediated muscle atrophy

Muscle atrophy induced by tail-suspension and denervation increases the expression of sarcolipin, which negatively regulates intracellular Ca^{2+} dynamics in muscle cells. To clarify the relationship of intracellular Ca^{2+} dynamics to muscle atrophy, we analyzed the changes in gene expression in denervated sarcolipin knock-out mice and denervated wild-type mice. We found that muscle atrophy in denervated sarcolipin KO mice was less than that in denervated wild-type mice. We are now examining the molecular mechanisms of this change.

Publications

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Department of Pathology
Division of Neuropathology

Masahiro Ikegami, *Professor and Director* Takahiro Fukuda, *Assistant Professor*

General Summary

Our research projects have concerned neurodegenerative disorders caused by the 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

Granulocyte colony stimulating factor producing solitary fibrous tumor

Introduction: Rare cases of granulocyte colony stimulating factor (G-CSF)-producing tumors exhibit leukocytosis due to G-CSF production and have an extremely poor prognosis. We histopathologically evaluated a G-CSF-producing solitary fibrous tumor. The patient was 39-year-old man who underwent resection of an extra-axial well-circumscribed right frontal convexity extracerebral dural-based tumor 60 mm in diameter. Biopsy of the tumor revealed a solitary fibrous tumor, characterized by staghorn vessels and a patternless architecture with hypercellular tumor cells, which had eosinophilic cytoplasm and a nucleus immunoreactive to signal transducer and activator of transcription 6. After surgery the patient was treated with radiation therapy. When the patient was 44 years old, 2 nodular tumors with a diameter of 10 mm appeared in the lung. At the age of 49 years, the patient complained of fever and abdominal pain and was found to have multiple metastatic tumors in the lungs, liver, kidneys, and adrenal gland. The white blood cell count was 70,500/μL (it had been 7,400/μL 5 years earlier), and the serum G-CSF concentration was 283.0 pg/mL. Biopsies of the liver and kidney tumors revealed a metastatic solitary fibrous tumor. Two weeks after being admitted to the hospital, the patient died of multiorgan failure. Tumor cells obtained when the patient was 39 and 49 years old expressed G-CSF, G-CSF receptor, and B-cell lymphoma 2 (bcl-2). The diagnostic criteria for G-CSF-producing tumors include: (1) a marked increase in leukocyte count, (2) elevated G-CSF activity, (3) a decreased leukocyte count following tumor resection, and (4) verification of G-CSF production in the tumor. This case fulfilled the criteria for G-CSF-producing tumors except for criteria 3. Expressions of G-CSF, G-CSF receptor, and bcl-2 in this tumor suggest that G-CSF itself has an effect on tumor cell growth and the inhibition of apoptosis. The prognosis of patients with G-CSF-producing tumors is usually poor, regardless of the primary organs. We should evaluate G-CSF and G-CSF receptor in solitary fibrous tumors.

Increase in messenger RNA level of subunit c of mitochondria ATP synthase in the central nervous system in a mouse model of prosaposin deficiency disease

Introduction: The pathophysiological changes of the central nervous system (CNS) in prosaposin knockout mice accompanied the degeneration of neurons and axons with organelle changes and the activation of ubiquitin-proteasome and autophagy-lysosome systems. In the CNS of prosaposin knockout mice 9 to 31 days old, the number of subunit c of mitochondria ATP synthase (SCMAS)-immunoreactive neurons increased in proportion to the number of amino-cupuric-silver-impregnated neurons. The accumulation of SCMAS might be induced by an increase in transcription of SCMAS messenger (m) RNA or a depletion of SCMAS degradation. This study investigated the mRNA level of SCMAS in the CNS of prosaposin knockout mice.

Material and methods: We analyzed the level of alternatively spliced SCMAS mRNA variants (ATP5G1, AT5G2, ATP5G3a, and ATP5G3b) in the CNS of wild-type and prosaposin knockout mice 9 to 29 days old with the real-time cycleave polymerase chain reaction using specific primers and probes.

Results: The levels of alternatively spliced SCMAS mRNA variants (ATP5G1, AT5G2, ATP5G3a, and ATP5G3b) all showed the same tendency. In wild-type mice, the level of SCMAS mRNA decreased in proportion to age. On the other hand, the level of mRNA increased in proportion to age in prosaposin knockout mice.

Discussion: SCMAS is a candidate for amino-cupric-silver-impregnated material in the CNS of prosaposin knockout mice. We reported the accumulation of SCMAS in the neuronal cytoplasm of neuronal ceroid lipofuscinoses, Niemann-Pick disease type C, Fabry disease, mucolipidoses, methylenetetrahydrofolate reductase deficiency, and mucopolysaccharidoses types I, II, and VII. In the CNS of prosaposin knockout mice, the increased level of alternatively spliced SCMAS mRNA variants would induce the accumulation of SCMAS-immunoreactive cells in proportion to an age of 9 to 29 days. The reason for increased SCMAS mRNA transcription is still unknown. The increase of ATP synthase might be induced by the depletion of ATP because of organelle dysfunction and the activation of ubiquitin-proteasome and autophagy-lysosome systems. We should investigate whether ATP decreases and whether SCMAS degradation is depleted in prosaposin knockout mice.

Publications

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Department of Orthopaedic Surgery
Division of Sports Medicine

Keishi Marumo, *Professor* Hiroki Funasaki, *Professor*

General Summary

Clinical Research
The ongoing research in our department concentrates on competitive athletes (including professional athletes), amateur athletes who include sports activities in their daily lives, and young athletes engaged in school sports clubs or dedicated to training within sports clubs.

Research Activities

Evaluation of arthroscopic surgery for sports-related injuries of the elbow joint
We examined the results of arthroscopic surgery in 20 patients who had had sports-related injuries of the elbow joint. The injuries were 10 cases of osteochondritis dissecancs (OCD) and 10 cases of osteoarthritis (OA). The average age of the patient during surgery was 15 years in the OCD group and 44 years in the OA group. At the final follow-up examination, the range of motion had improved from the preoperative evaluation range by 13 degrees in the OCD group and by 10 degrees in the OA group. Early pain recovery was obtained, and no patient had had intraoperative or postoperative complications. All patients returned to their previous sports activities. Arthroscopic surgery was useful for resection of the free body, synovia, and the bone spur in patients who had OCD and OA of the elbow joint.

Incidence of injuries in professional soccer players: Role of the team physician
We investigated the incidence of injuries and evaluated injury characteristics and treatment difficulties in athletes who had played soccer in the Japan professional league (the J League) for 3 years. Seven players underwent surgery for fractures (3 players), for tears of the anterior cruciate ligament (2 players), or for detachments of the proximal hamstring tendon (2 players). In 2017, 41 injuries, more than half of the total of 75 injuries, consisted of muscle-tendon injuries, including muscle strain. Difficulties of treatment included partial avulsion of the proximal hamstring tendons, intratedinous tendon tears of the rectus femoris muscle, and damage of the musculotendinous junction of the gastrocnemius muscle. Our data suggests the necessity of establishing a proper diagnosis, treatment method, and rehabilitation protocol to allow players with such injuries to quickly return to their preinjury sports activities.

A case of pubic apophysitis developed in a youth soccer player
We report a case of pubic apophysitis that developed in a 17-year-old soccer player. Although X-ray images and magnetic resonance imaging scans had no abnormal findings,

a computed tomography examination showed asymmetrical epiphyseal centers at the pubic tubercle. The patient returned to play after 3 weeks of conservative treatment. Pubic apophysitis, which presents with symptoms similar to those of groin pain syndrome, has been known as a new concept first described in 2015. We need to understand its pathology to be able to differentiate it from other injuries causing groin pain that occur in junior high school and high school athletes.

Open reduction and internal fixation with autogenous bone graft for pseudoarthrosis of the old ischial tuberosity avulsion fracture: A case report
We report on a 27-year-old baseball player who underwent surgery for pseudoarthrosis of an ischial tuberosity avulsion fracture that had occurred 13 years earlier and for whom satisfactory results were obtained. Operative findings showed marked instability in the pseudoarthrosis region. Pseudoarthrosis was curetted and fixed with screws and an autogenous bone graft. Three months after the operation, complete bone union was shown with X-ray images. The patient was able to return to play 6 months after the operation. To our knowledge, only a single report has previously appeared in the literature of a case with symptoms that appeared in a patient in his twenties and originated from pseudoarthrosis of an ischial tuberosity avulsion fracture. In our patient, open reduction and internal fixation with an autogenous bone was effective to stabilize pseudoarthrosis.

A rare case of quadratus lumborum muscle strain that developed in a youth soccer player
We report a rare case of quadratus lumborum muscle strain that occurred during a soccer game. The patient was a 16-year-old boy playing for a youth soccer team. When he was dribbling the ball with another player on his left side, he passed the ball forward and fell on his right foot. At that time, he felt severe pain in the right side of the back. Two weeks after the injury, motion pain and tenderness of the back were not remarkable, and magnetic resonance imaging showed improvement of the muscle strain. He returned to play soccer 3 weeks after the injury. To our knowledge, a case of quadratus lumborum muscle strain that occurred during a sport activity has not previously been reported.

Publications

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Department of Innovative Interventional Endoscopy Research

Hisao Tajiri, *Professor* Masato Mitsunaga, *Assistant Professor*

General Summary

This department was established in April 2015, with the aims of establishing new methods of endoscopic diagnosis and treatment and developing new apparatuses and with the purposes of supporting and teaching to standardize endoscopic medicine in Japan and in foreign facilities.

Research Activities

Endoscopic submucosal dissection (ESD), which was developed in Japan, has been followed by various improvements for safety, promptness, and accuracy. Following ESD, new minimally invasive endoscopic treatments, such as endoscopic full thickness resection and endoscopic treatment applying robotic technology, are being developed. Minimally invasive endoscopic treatments, which are less of a burden to patients, make a substantial contribution. As the social demands for endoscopic medicine grow, new endoscopic treatment methods and new instruments are being developed. This department plays several roles, such as performing relevant research studies and developing new educational methods for training physicians to perform endoscopy in Japan, other parts of Asia, Russia, the Middle East, and South America.

Endoscopic optical molecular imaging for cancer

Molecularly targeted therapies, such as monoclonal antibodies, have been widely used for various cancers and have improved patient outcomes. Photoimmunotherapy (PIT) is a new class of molecularly targeted cancer theranostics which employs monoclonal antibodies conjugated to a photosensitizer, IRDye 700DX (IR700). The photosensitizer is activated by focal near-infrared (NIR) light irradiation, leading to necrotic cell death by cell membrane rapture, where antibody-IR700 conjugates bind to target membrane proteins specifically. A global phase 3 clinical trial of PIT for patients with recurrent cancer of the head and neck has been conducted with a monoclonal antibody (mAb) against epidermal growth factor receptor, a cetuximab-IR700 conjugate. In this study, we developed a new type of PIT agent targeting vascular endothelial growth factor receptor 2 (VEGFR-2), which is expressed on the vascular endothelium in a tumor, and evaluated the feasibility by comparing conventional membrane-targeted PIT in vitro and in vivo. Human epidermal growth factor receptor 2 (HER2)-positive human gastric cancer cells of the NCI-N87 cell line were used for the experiments. Conjugated to IR700 were HER2-targeting trastuzumab and VEGFR-2-targeting DC101. Cells were treated with mAb-IR700 conjugates and then, after being washed, with NIR light irradiation. A mouse model of a tumor xenograft was created for in vivo PIT. Tumor-bearing mice were randomized and treated

under anesthesia with mAb-IR700 conjugates followed by NIR light irradiation. The PIT utilizing DC101-IR700 conjugates did not induce a phototoxic effect in vitro because of the absence of membranous expression of VEGFR-2 in NCI-N87 cells, whereas PIT utilizing trastuzumab-IR700 conjugates induced a rapid phototoxic effect because of the strong membranous expression of HER2 in NCI-N87 cells. In contrast, antitumor effects were observed in NCI-N87 xenograft tumors in vivo utilizing both DC101- and trastuzumab-IR700 conjugates followed by NIR light irradiation. Because VEGFR-2 is upregulated in many types of solid cancer, this method might be considered as applicable to various types of cancer.

Evaluation of 0.6% sodium alginate as an endoscopic submucosal injectant for esophageal and gastric ESD

Because ESD requires a high level of skill and is targeted at larger lesions, the risks of prolonged treatment and increased bleeding and perforation might increase. From these points of view, to perform ESD more easily and safely, maintaining a sufficient mucosal raise for the lesion and its surroundings is important. Currently, 0.4% sodium hyaluronate solution is widely used as an endoscopic submucosal injectant, but a problem is that the price of insurance coverage is high. Therefore, we have focused on sodium alginate with viscosity as an endoscopic submucosal injectant that is less expensive and does not impair mucosal raise as much as the 0.4% sodium hyaluronate solution does. In conducting ESD for patients with neoplastic lesions in the esophagus and gastric mucosa, we participated in a multicenter study as a control group using 0.4% sodium hyaluronate solution for the efficacy, safety, and usefulness as an endoscopic submucosal injectant. In this study, as an advisor to clinical trials, Tajiri has taught much from protocol construction to publication of the articles. Of a total of 130 cases, 65 were in the test group and 65 were in the control group. Sodium alginate is an inexpensive natural material widely used in various fields, and it became clear that the 0.6% sodium alginate solution, whose physical properties were adjusted in this study, has excellent mucosal raising ability without inferring with catheter passage.

Development of automatic insertion endoscope equipment

We are developing a motorized spiral enteroscope. This enteroscope is unique because it incorporates a user-operated motor to rotate the power spiral tube, which is mounted on the endoscope's insertion tube and relies primarily on the pleating of the small bowel onto the scope with minimal pushing.

Representatives from Japan, Europe, and the United State repeated experiments in vivo and in vitro with medical device manufacturers, and clinical trials were conducted for the first time in Europe. As a representative of Japan, Tajiri was in charge of animal experiments and has participated as an advisor to clinical trials in Europe. This instrument is a safe and effective tool for the diagnosis and treatment of small bowel disease and has been available in Europe since 2018. We believe this instrument will become a standardized enteroscope. In addition, the procedure time is shorter than that of the conventional scope, and we anticipate that this technology will be applied to colonoscopy.

Educational Activity

Since 2014 the Japan Gastroenterological Endoscopy Society has been leading hands-on courses in China, and in 2018 we visited Dairen and Xiamen, where we gave lectures and performed therapeutic manipulation with animal models and live demonstrations. The young physicians being trained made remarkable progress in early cancer detection and diagnosis and endoscopic treatment. In Russia, Vietnam, Myanmar, Indonesia, and Brazil, we have been conducting similar activities. Hands-on courses have already been held in rural regions of Japan. We have visited and will visit the regions, to develop facilities where endoscopic diagnosis and treatment can be standardized. We will continue to conduct these supportive activities.

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Research Center for Medical Sciences
Division of Gene Therapy

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

General Summary

The optimal lentivirus vector was selected in hematopoietic stem cell (HSC)-targeted gene therapy using a lentivirus vector for mucopolysaccharidosis type II (MPS II), which was funded by the Agency for Medical Research and Development. In another MPS II gene-therapy project funded by this agency, significant reductions of accumulated compounds in the brain were observed with intravenous administration of adeno-associated virus (AAV) vectors. In addition, we have started to develop gene therapy for GM1 gangliosidosis and an artificial intelligence (AI) study of Fabry disease.

Research Activities

Summary of proceedings in ex vivo HSC gene therapy for MPS II
To develop gene therapy for MPS II, we have reported efficient substrate reduction with a lentiviral vector in the central nervous system (CNS) of a mouse model of MPS II (2015). We have changed the construction of this vector to the 3rd generation, similar to a vector needed for clinical trials, and succeeded in substrate reduction in important organs and behavioral tests. We applied for a patent of these results and are preparing for nonclinical tests and clinical trials.

Ex vivo HSC gene therapy for a mouse model of MPS II
We compared several lentiviral vectors with a different promoter (phosphoglycerate kinase, CD11b, or MPSV LTR, NCR deleted, dl587 PBS [MND]) for the treatment of a mouse model of MPS II. The HSC was transduced by each lentiviral vector and transplanted to the mice. Lentiviral vectors with the MND promoter achieved high levels of iduronate 2-sulfatase (IDS) enzyme activity and a significant reduction of glycosaminoglycan (GAG) storage, both in peripheral tissue and the CNS.

$CD34^+$ HSC gene therapy for MPS II
This year we optimized the lentiviral transfection method for $CD34^+$ cells *in vitro* and transplanted the cells to NOG-MPS II mice. With the newly established method the copy number of $CD34^+$ cells was approximately 2.5 times as high as with our previous method. The analysis of $CD34^+$ cell-transplanted mice is ongoing. Additionally, we have developed an antitransferrin antibody-fused IDS, which has high cell-penetrating ability.

Lethal preconditioning needed to ameliorate CNS involvement of MPS II
We have previously reported that HSC-mediated gene therapy using lentiviral vector ameliorates CNS involvement. We compared 3 conditioning procedures before gene ther-

apy. The results showed that only a lethal conditioned group achieved behavior correction providing an elevation of IDS activity and a reduction of GAG in CNS tissue.

AAV vector-based gene therapy for murine Fabry model
We intrathecally injected the rAAVPHP.eB vector encoding Gla into a mouse model of Fabry disease and bred the mice for 7 months. In the AAV-gene therapy group, the dorsal root ganglion tissue Gla enzyme activity level was 88% greater than in wild-type control mice. With hot-plate analysis at 52°C, the AAV-gene therapy group showed a significant improvement in thermal hypoalgesia.

Development of gene therapy for GM1 gangliosidosis
GM1 gangliosidosis is characterized by deficient activity of β-galactosidase, resulting in accumulation of GM1 ganglioside and causing CNS disease. We aimed to develop HSC-targeted gene therapy. We constructed a lentiviral vector expressing β-galactosidase under control of a phosphoglycerate kinase promoter and performed gene therapy in a mouse model of GM1 gangliosidosis. Increases in enzyme activity and vector copy number were observed in peripheral organs but not in the brain.

AAV-mediated gene therapy for MPS II
We intravenously administered the amount of 1.0×10^{11} vector genomes/mouse of the AAV9 vector expressing the optimized IDS gene to 8-week-old MPS II mice. The results showed a significant elevation of IDS activity and a reduction of GAG accumulation in homogenates of the cerebrum and cerebellum 16 weeks after treatment.

Gene therapy for bone complication of MPS II, gene therapy for Krabbe disease, and the construction of an AI-mediated database of Fabry disease
We investigated the bone complications in MPS II mice, detected density and strength greater than those in normal mice, and succeeded in decreasing the changes with *ex vivo* gene therapy. For Krabbe disease, we studied the effects of neonatal *in vivo* gene therapy with lentiviral vectors and succeeded in substrate reduction in the CNS, a delay of the onset time point, and an improvement of survival rate. We planned the study for Fabry disease, aiming at a new diagnostic system by AI-mediated deep learning of the relation between secondary genomic change and magnetic resonance imaging or blood analysis, and applying to the institutional review board.

New strategy of gene therapy with suppression of lysosome enzymes
Suppression of lysosome function is expected to be a new strategy for treating chemoresistant cancer. We hypothesized that down-regulation of lysosomal enzymes induces lysosomal dysfunction and enhances chemosensitization. We now knock down several lysosomal enzymes and are clarifying the role of each enzyme's activity in cancer.

Publications

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Research Center for Medical Sciences
Division of Oncology

Sadamu Homma, *Professor and Director*
Shigeo Koido, *Associate Professor*
Yuko Kamata, *Assistant Professor*
Yasuharu Akasaki, *Associate Professor*
Masaki Ito, *Assistant Professor*

General Summary

The aim of our research is to develop and establish novel cancer therapies. Concepts of new anticancer therapy generated from unique ideas of the researchers would be verified by basic and clinical studies so that such concepts can be applied to the clinical cancer treatment. Most of our research has been based on antitumor immunity.

Research Activities

High blood levels of soluble OX40, an immune costimulatory molecule, indicate reduced survival in patients with advanced colorectal cancer

The interaction between OX40 (CD134) on T cells and the OX40 ligand (OX40L) on antigen presenting cells (APCs) is a pivotal step for activating T cells and promoting antitumor immunity. However, soluble OX40 (sOX40) in blood is thought to suppress T cell activation by blocking OX40/OX40L interaction. Here, we investigated the association between blood sOX40 levels and the clinical characteristics of patients with advanced colorectal cancer. Blood was collected from 22 patients with advanced colorectal cancer. Blood sOX40 levels were determined with enzyme-linked immunosorbent assay. Blood sOX40 levels were positively correlated with the blood levels of carbohydrate antigen 19-9, carcinoembryonic antigen, C-reactive protein, and soluble programmed cell death ligand-1 but were negatively correlated with the blood levels of albumin. Importantly, according to both univariate and multivariate analyses, high blood sOX40 levels were significantly correlated with a reduced survival time. High blood levels of sOX40 were possibly associated with the suppression of antitumor immunity by sOX40.

Induction of Wilms tumor 1-specific cytotoxic lymphocytes by the artificial antigen vaccine

From a functional viewpoint, vaccine adjuvants are classified into 2 types: "physical adjuvants" increase the efficacy of antigen presentation in APCs, and "signal adjuvants" induce the maturation of APCs. By using ovalbumin as a model antigen, we have previously demonstrated that a physical adjuvant can be encrypted into proteinous antigens (F37A) by creating artificial proteins with motif-programming (Ito M et al, PLOS ONE 2014, 2017). In the present study, we replaced the MHC class I and class II epitope peptide motifs of ovalbumin in F37A with those of the Wilms tumor 1 (WT1) epitopes to construct a WT1 artificial antigen, WT1-C2. When WT1-C2 was incubated with human peripheral blood mononuclear cells, the antigen was endocytosed to dendritic cells (con-

firmed by flow cytometry) to induce WT1-specific cytotoxic lymphocytes (CTLs) (confirmed by tetramer assay). The induced CTLs were cytotoxic to T2-A24 lymphoblastoid cells pulsed with WT1 epitope peptide. Therefore, F37A can be used as a framework protein to construct a physical adjuvant free-artificial antigen by substituting the ovalbumin epitopes in F37A with another peptide motif, which induces CTLs against target cells.

Search for the immunogenic mutation-derived antigens of human malignant brain tumors
Dendritic/tumor fusion cell vaccine therapy has been performed for the treatment of patients with glioma at The Jikei University Hospital. Variant peptides generated by the gene mutation of glioma cells might be functioning as target tumor antigens in fusion cell vaccine therapy. We have performed whole exome and whole transcriptome analysis of gliomas from adult patients treated with the fusion cell vaccine and have identified several candidates for a novel mutation-derived antigen. A clinical trial of the fusion cell vaccine to pediatric brain tumors was start this year. Several candidate mutation-derived antigens were identified by the analyses in pediatric brain tumors. Furthermore, glioma stem-like cells induced from original glioma cells *in vitro* are often available in generation of the fusion cell vaccine. The use of glioma stem-like cells as a fusion partner of dendritic cells might make the fusion cell vaccine more effective. Glioma stem-like cell-specific mutated antigens were searched for, and several candidates were found.

Publications

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Research Center for Medical Sciences
Division of Molecular Immunology

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

General Summary

Our research interests have focused on the analysis of the basic immune system, which protects us from a number of diseases, and of immune disorders, such as hypersensitivity diseases and autoimmune diseases.

Research Activities

Involvement of interleukin 31 receptor A in morphine-induced itching and antinociception in mice

Morphine is an effective analgesic for the treatment of severe pain, but it can cause itching in patients. The present study examined the possible involvement of interleukin (IL) 31 receptor A (IL-31RA) in morphine-induced itching and antinociceptive effects in IL-31RA-deficient mice and wild-type mice. The present findings suggest that IL-31RA plays a significant role, perhaps in sensory neurons or the spinal cord or both rather than in the brain, in the modulation of morphine-induced itching and antinociception. Here, we have demonstrated that IL-31RA is a possible common mediator of itching and the antinociception of morphine. Therefore, IL-31RA might be a noteworthy target for considering the novel mechanism of itch and pain signaling affected by morphine.

Evaluation of basophil activation caused by transgenic rice seeds expressing whole T-cell epitopes of the major Japanese cedar pollen allergens

Japanese cedar (JC) pollinosis is a serious type I allergic disease in Japan. Although subcutaneous immunotherapy and sublingual immunotherapy have been used to treat JC pollinosis, high doses of allergens might cause immunoglobulin E-mediated allergic reactions. Developed as candidates for oral immunotherapy are transgenic rice seeds that contain genetically modified Cry j 1 and Cry j 2, the 2 major allergens of JC pollen. To verify the allergenic safety of transgenic rice seeds, we investigated with the basophil activation test the percentage of activated basophils induced by transgenic rice seed extract. Blood samples were collected from 29 patients with JC pollinosis. Transgenic rice seed extract, nontransgenic wild-type rice seed extract, and Cry j 1 and Cry j 2 were mixed with the blood via reagents. The percentage of activated basophils was assessed via the expression of CD203c, a basophil activation marker.

The percentage of activated basophils after stimulation with transgenic rice seed extract was significantly lower than that induced by the mixture of Cry j 1 and Cry j 2. Transgenic rice seed extract caused little activation of basophils in patients with JC pollinosis compared with that induced by the mixture of Cry j 1 and Cry j 2. Furthermore, the per-

centage of activated basophils did not differ significantly between transgenic rice seed extract and wild-type rice seed extract.
Regarding the risk of immunoglobulin E-mediated allergic reactions, the results show that transgenic rice seeds would be much safer than conventional immunotherapies with crude antigens, including Cry j 1 and Cry j 2, and might be as safe as wild-type rice seeds.

Development of vaccination to induce cytotoxic T lymphocytes against tumor-specific antigens

A vaccine that raises specific cytotoxic T cells against tumors or pathogens is the convincing approach to overwhelm these diseases. On the basis of a previous study, we have developed a new liposome-based adjuvant to induce cytotoxic T lymphocytes (CTLs) by mixing protein antigens and adjuvant before administration. To use this vaccine to treat cancer, further analysis was carried out, and these vaccine-induced helper T type 1 cells efficiently shifted the immune response, but inducing CTLs against cancer was difficult. To prime CTL induction, we compared several candidates for suicide gene therapy. With suicide gene therapy against colon tumors, an efficient tumor vaccine was acquired to reject 1 million tumor cells implantation. Also, the cryoimmunization of tumor expression ovalbumin as a reporter was confirmed to have induced specific CTLs. With these results, we are developing a vaccine to induce CTLs to suppress tumor recurrence.

Publications

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Research Center for Medical Sciences
Division of Medical Engineering

Masayuki Yokoyama, *Professor and Director* Kouichi Shiraishi, *Associate Professor*

General Summary

The Division of Medical Engineering aims to provide new and essential techniques for developing medical treatment. We have focused on 2 projects. One project is to develop polymer or polymeric micelle drug carrier systems for efficient therapeutic and diagnostic treatment. An example of our drug carrier systems is a polymer-based magnetic resonance imaging (MRI) contrast agent carrier system for the precise diagnosis of diseases. The polymer-based MRI contrast agents possess great potentials for disease-specific MRI. However, a drawback of the polymer-based MRI contrast agents is a risk of the release of free gadolinium (Gd) ions from Gd-chelates, which is due to the long half-lives of the polymer-based MRI contrast agents. Therefore, we designed a new stable polymer-based Gd-chelate for disease-specific MRI and have synthesized the polymer-based Gd-chelate. The other project has focused on immunogenicity issues of poly(ethylene glycol) (PEG), which has been the most commonly used polymer for biopharmaceutics, cosmetics, and foods. Because PEGs are considered safe, nonimmunogenic or weakly immunogenic, and biocompatible, PEG conjugation (PEGylation) is the most common technique for therapeutic proteins. However, PEGylated materials reportedly induce anti-PEG antibodies. We have examined PEG-related antibody responses in terms of the chemical structures of PEG-conjugates and have suggested an interfering concept. The study revealed the immunogenic characteristics of PEGs.

Research Activities

Development of Gd-chelates for the safe use of polymer MRI contrast agents

The MRI contrast agents are diagnostic agents that enhance MRI signals of water by paramagnetic metal ions, such as Gd ions, and iron ions and have been used clinically. At present, MRI contrast agents are low-molecular-weight Gd-chelates, which have short half-lives. On the other hand, in several recent studies, repeated injections of specific Gd-chelate-based MRI contrast agents have caused nephrogenic systemic fibrosis in patients, who exhibited low renal function. A possible reason this fibrosis develops is the fibrillization of free Gd ions, which are released from unstable Gd-chelates.

Regarding the functions of MRI contrast agents, we have shown attractive functions of polymer-based MRI contrast agents for the precise diagnosis with MRI. However, polymer-based MRI contrast agents have much longer half-lives than do low-molecular-weight Gd-chelates. Although the long half-lives are a reason these polymer-based MRI contrast agents have attractive functions, they also cause our bodies to be long exposed to these agents. Therefore, the Gd stability in our body is an issue we consider important.

To avoid the release of free Gd ions from Gd-chelates, we have developed new polymer-

based MRI contrast agents exhibiting stable Gd-chelates. We used a 1-(1-carboxy-3-carbo-*tert*-butoxypropyl)-4,7,10-(carbo-*tert*-butoxymethyl)-1,4,7,10-tetraazacyclododecane group for conjugation to polymers, which were deprotected to form a 1,4,7,10-tetraazacyclododececane, 1-(glutaric acid)-4,7,10-triacetic acid (DOTAGA) chelate group. The DOTAGA chelate group possesses 8-coordination after conjugation, whereas the 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacatic acid (DOTA) chelate group possesses 7-coordination after conjugation. We synthesized poly(glutamic acid)-based MRI contrast agents possessing the DOTAGA chelate group. After we prepared new MRI contrast agents, we have developed a method to evaluate the stability of Gd ions by means of a high-performance liquid chromatography system. We compared several low-molecular-weight Gd-chelates and poly(glutamic acid)-based Gd-chelates. We found no significant differences of Gd release from Gd-chelates in a 50% serum condition. In contrast, a possible reason for the release of Gd ions is the interaction of Gd ions with phosphate ions. Therefore, we examined the mixture containing 50 mM phosphate buffer and examined the stability of Gd-chelates. We observed the significant release of Gd ions from a low-molecular-weight Gd-chelate. The poly(glutamic acid)-based Gd-chelates exhibited no significant differences in the release of Gd ions, as compared with diethylenetriaminepentaacetic acid gadolinium (Gd-DTPA). On the other hand, a previously prepared polymeric micelle MRI contrast agent, which possesses a 7-coordinated DOTA chelate group, exhibited the highest stability of Gd ions. We have started developing a new polymeric micelle MRI contrast agent possessing an 8-coordinated DOTAGA chelate group.

Immunogenicity issues of PEG

We have studied the immunogenicity of synthetic polymers called PEG-conjugates. A conjugation technique of PEG to proteins, nanoparticles, and bio-surfaces is a simple technique known as PEGylation. PEGylation to proteins produces bio-inert surfaces, and as a result, reduces the immunogenicity of proteins and improves their pharmacokinetics. However, in patients to whom PEG-protein drugs have been repeatedly administered, anti-PEG antibodies have reportedly been induced and have become a serious issue for medical treatments. Because PEGylated proteins are captured by anti-PEG antibodies, the therapeutic efficacy of PEGylated proteins has been decreased. In fact, nonresponsive patients, who have been treated with PEGylated uricase, exhibited strong anti-PEG antibodies' responses, whereas responsive patients exhibited very weak anti-PEG antibodies' responses. Many PEGylated therapeutic proteins have been examined in clinical trials. However, because PEGylated therapeutic proteins induce anti-PEG antibodies, each PEGylated protein has immunogenic potential. As by PEGylated proteins, anti-PEG antibodies are reportedly induced by PEGylated nanoparticles (liposomes, micelle, and other nanoparticles). The antibodies in reported cases are most often anti-PEG immunoglobulin M (IgM) antibodies. Although PEGs are thought to be nonimmunogenic or weakly immunogenic polymers and are used to create bio-inert surfaces for research, these facts indicate that PEGs induce specific immune responses.

We have used various PEG-block copolymers to reveal PEG-related immunogenicity and have optimized anti-PEG IgM responses by the use of PEG-poly(β-benzyl L-aspartate)

block copolymer (PEG-PBLA), and derivatives of PEG-PBLA (PEG-P(Asp-Bzl)). From our mechanistic study of anti-PEG IgM behaviors, we have suggested an interfering concept for reducing anti-PEG IgM responses and designed new PEG-block copolymers possessing an intermediate block. The prepared new PEG-block copolymer micelles have shown in various doses a weak or nonexistent anti-PEG IgM response in vivo. The result indicates that our interfering concept works under in-vivo conditions.

We have started a new project, which is funded by the Japan Society for the Promotion of Science (Fund for the Promotion of Joint International Research). The project is based on an earlier finding that the interfering concept helps to reduce specific antibody responses; the project focuses on the immunogenicity of PEGylated proteins. We collaborate with 2 international researchers to promote our project. In the first year of the project, we started to prepare new PEGylated proteins, which exhibit nonexistent or extremely weak anti-PEG antibody responses.

Reviews and Books

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Research Center for Medical Sciences Division of Ultrasound Device Development and Application (DOUDA)

Norio Nakata, *Associate Professor and Director* Zuojun Wang, *Assistant Professor*

Research Activities

Education/Research outline

1. Development of a decision-supporting system for breast ultrasonography using deep learning

The purpose of this study is to develop a decision-supporting system for breast ultrasonography using deep learning, which is machine learning technique. The goal of this system is a classification tool between benign and malignant breast mass lesions. Required by this study are at least 1,000 cases of supervised data sets, including breast ultrasonographic images and pathologic diagnosis results. This study has already been approved by The Jikei University Ethics Committee. We are preparing to install the deep learning program and to experiment on artificial intelligence (AI) tests. With this study we are expecting to improve the diagnostic efficiency of breast ultrasonography for diagnostic radiologists.

2. Improvement of computer hardware and software environment for AI development

In Japan Agency for Medical Research and Development, 2017: "Clinical research etc. ICT infrastructure building · AI implementation research project" Adoption of secondary public offering (medical productivity revolution realization project), utilization of AI (research representative: Masatoshi Kudo, president of the Japan Society of Ultrasonic Medicine),

As a research contributor, Norio Nakata has developed ultrasonographic image processing and prototype diagnosis support. In this first year, to study the AI transfer learning effect with images of other areas, we developed an AI algorithm for judging diseases of the chest with chest X-ray images (more than 100,000) published at the NIH to prepare computer hardware and software for AI development.

3. Education and awareness activities to promote AI utilization in diagnostic radiology

In the Japan Society of Ultrasonic Medicine and the Japan Radiological Society, we conducted educational and awareness activities to explain to academic members (ultrasonic specialists and radiologists) the principle and the near future use of AI for imaging support. Norio Nakata wrote and published its contents in the journal of the Japanese Society of Nephrology and the Journal of Medical Image Information Society.

4. In vitro study of a method of quickly reopening acutely occluded vessels with ultrasound and microbubbles

Early reopening of occluded blood vessels is the most effective treatment for acute cerebral infarction. Transcranial ultrasound, alone or in combination with microbubbles, has been shown to promote the thrombolytic efficiency of recombinant tissue plasminogen

activator (rt-PA). However, thrombolytic therapy has frequently failed for completely occluded cerebral arteries, likely because rt-PA has difficulty reaching the thrombus site. We theoretically examined and demonstrated that the combined use of ultrasound and microbubbles has the effect of transporting rt-PA over a long distance, in addition to locally promoting the thrombolytic effects of rt-PA. In this study, we are conducting research on the existence and amplitude of this transportation effect and its relationship with various parameters of ultrasound through in-vitro experiments.

5. Study of method of preventing vascular re-occlusion with ultrasound

After thrombolytic therapy with intravenous rt-PA, blood vessels are frequently re-occluded. Because anticoagulation therapy is prohibited within 24 hours after rt-PA treatment, vascular re-occlusion is a fatal problem. Together with Lecturer Sawaguchi (Faculty of Biomedical Engineering, Toin University of Yokohama), we have conducted research on the thrombus growth control effect of noninvasive ultrasound with an in-vitro clot growth model. This study showed that noninvasive ultrasound irradiation can control thrombus growth. Safe and simple ultrasonic irradiation can be used to prevent re-occlusion after rt-PA treatment for acute cerebral infarction. Additional advanced basic research studies are being conducted to allow clinical application.

Publications

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Research Center for Medical Sciences
Division of Neuroscience

Fusao Kato, *Professor and Director*

General Summary

The mission of the Department of Neuroscience has 3 parts. The first part is to advance and promote research on the roles played by the brain neuronal network in various aspects of biological functions in health and disease. The functions of the central nervous system undergo plastic, adaptive, and allostatic changes that play essential roles in the development and persistence of many diseases caused by the dysfunction of peripheral organs. An example is pain, which is a biologically necessary function that detects aversive situations in the body and urges reactions to improve the situation. At the same time, however, pain is highly distressing and disturbs the daily life and thoughts of patients. Despite the cause of pain often being in the periphery (but not always for chronic pain), the plastic changes in the brain, particularly in the regions of survival/defence, emotion, and cognition, alter the perception of pain and modulate the sensitivity of pain. These changes in perception and sensitivity affect the autonomic, endocrine, and cognatic systems. To identify the mechanisms underlying the plastic changes, we use electrophysiological, behavioural, imaging, and genetic tools in various models of diseases with pain.

The second part of the mission is to develop novel technologies that would help the first part be achieved. An example of a novel technology is a combination of chemogenetics and manganese-enhanced magnetic resonance imaging of the brain from models of chronic inflammatory pain. This approach allows the limitations of magnetic resonance imaging of the brain in animal models, such as the necessity of anesthesia, the lack of causality-related information, and the difficultly in imaging brain activity associated with spontaneous behaviour, to be surpassed.

The third part of the department's mission is to provide an opportunity for young researchers and students to participate in studies with updated technology and tools used in modern brain sciences and to experience the planning, practicing, summarizing, presenting, and publishing of novel findings. Because of the complexity of its targets and the extremely rapid progress being made, neuroscience is an advanced domain of integrative biology.

To achieve the department's mission, we use approaches at the molecular, cellular, synaptic, and network levels, including the patch-clamp recording of synaptic currents, the real-time imaging of intracellular Ca^{2+} concentration, and behavioral analyses combined with optogenetic and chemogenetics approaches in healthy animals and animal models of various diseases.

As of March 2019, the department is composed of 1 professor/director, 2 research assistant professors (Dr. Yukari Takahashi and Dr. Yae K Sugimura), 1 part-time lecturer, 8 Ph.D. students, 1 foreign visiting student (Iran), 1 research supporter, and many other visiting researchers. At The Jikei University we give lectures in neurophysiology and pro-

vide practical laboratory work to medical students and give lectures to graduate students. We also participate in a wide range of social activities, including being board members of scientific societies and members of the Science Council of Japan.

Research Activities

In the 2018 fiscal year, we have examined the following subjects.
1. Establishment of transgenic rats expressing cre recombinase under vesicular GABA transporters (VGATs) and dopamine-β-hydroxylase (DBH) promotors. These are now deposited at the National BioResource Project - Rats.
2. Identification of the role of the central amygdala in widespread hypersensitivity through selective activation and inhibition of GABAergic neurons using VGAT-cre rats and chemogenetics.
3. Identification of the role of endogenous noradrenaline released in response to light stimulation of terminals in the central amygdala arising from the nucleus of the solitary tract using DBH-cre rats and channelrhodopsin expression.
4. Analysis of the activation patterns of the lateral parabrachial nucleus and the basolateral/central amygdalae using c-Fos immunohistochemistry in a newly developed, formalin-induced model of latent inflammatory pain.
5. Analysis of the role of inflammatory factors in the plastic changes of the central pain network during the shift from acute pain to chronic pain.
6. Development of methods of selective gene expression in the trigeminal ganglion using adeno-associated viruses.
7. Evaluation of spontaneous/voluntary behaviors in animals with collagen-induced rheumatoid arthritis using a wheel-running paradigm and temperature-dependent choice.
8. Visualization of neuronal activation and dopamine receptor expression in the brain reward system in response to acute itch using multiple single RNA imaging.
9. Fast intracellular Ca imaging for comparing the neuroglial responses to exogenous oxytocin in the central amygdala of female mice from before pregnancy to before and after delivery.
10. Behavioral analysis of the relationships of the social rank order of individual mice to glucose tolerance and insulin release regulation.

Publications

Yamauchi N*[1]*, Takahashi D, Sugimura YK, Kato F, Amano T*[1]*, Minami M*[1] *(*[1]Hokkaido Univ).*** Activation of the neural pathway from the dorsolateral bed nucleus of the stria terminalis to the central amygdala induces anxiety-like behaviors. *The European Journal of Neuroscience.* 2018; **48:** 3052-61.

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Reviews and Books

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Research Center for Medical Sciences
Division of Clinical Pharmacology and Therapeutics

Shigeru Kageyama, *Professor and Director* Akihiro Ohnishi, *Professor*

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 some other departments of clinical pharmacology in Japan focus on registration trials, particularly phase I trials. Because a clinical laboratory where we had performed many human pharmacological studies became unavailable in 2003, we shifted our research from human studies to multicenter clinical trials and pharmacoepidemiological studies.

Research Activities

We have performed a large-scale pharmacoepidemiological study of the safety of statins. Because the study took a long time to complete, we organized a research group comprising academic and industrial organizations (Japanese Society for Pharmacoepidemiology, Japanese Society of Clinical Pharmacology and Therapeutics, Japan Association for Medical Informatics, Japan Society of Clinical Trials and Research, Federation of Pharmaceutical Manufacturers' Associations of Japan, Pharmaceutical Research and Manufacturers of America, and European Federation of Pharmaceutical Industries and Associations, Japan) to make postmarketing studies more efficient by utilizing the Standardized Structured Medical-record Information eXchange (SS-MIX). The SS-MIX system was started in 2006 as a project supported by the Ministry of Health, Labour and Welfare to promote the exchange of standardized medical information. The SS-MIX system will increase the efficiency of pharmacoepidemiological studies by identifying "new users" who have started using the drug after a period of nonuse. The "new user" design is often essential for obtaining unbiased results. In the 3 hospitals affiliated with The Jikei University (Katsushika Medical Center, Daisan Hospital, and Kashiwa Hospital), where electronic medical record systems have already been installed, we collected prescription and medical test data. As of January 2016, prescription and medical test data have been incorporated into SS-MIX. In January 2018 data from The Jikei University Hospital was finally incorporated into SS-MIX storage, a large scale data set of various diseases from all 4 hospitals became available. We introduced this system to Department of Dermatology and to all the divisions of Department of Internal Medicine. Several studies using the SS-MIX system are in progress.

To raise the literacy of clinical trials among researchers, we held the "Clinical Trial Seminar" in October 2018. The theme was "Big Data in Cardiovascular Diseases and Clinical Innovation Network."

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. Clinical trials are now facilitated by 10 clinical research coordinators (CRCs), of whom 2 have also been involved in monitoring. The CRCs have started to help with both registration trials and investigator-initiated trials. The CRCs have been introduced into all registration trials since 2004; the quality and speed of these trials have been much improved.

Investigator-initiated registration trials which were managed by other medical institutions have been conducted so far. Since 2017 an investigator-initiated registration trial which was designed and planned by an investigator of The Jikei University has been conducted. In this trial data management was outsourced to a contract research organization.

We have also supported investigator-initiated clinical trials in collaboration with the Clinical Research Support Center. Details are described in the Clinical Research Support Center section of this annual review.

Research Center for Medical Sciences
Division of Molecular Epidemiology

Mitsuyoshi Urashima, *Professor and Director*

General Summary

Despite having the same disease diagnosis, some patients may be cured, but some may not. This difference cannot be understood with experimental medicine. On the other hand, the answer might not be provided by clinical practice. We combined molecular biology and epidemiology to create the Division of Molecular Epidemiology, to clarify the etiology of diseases and to predict factors affecting survival.

Research Activities

The Jikei clinical research course

We have held 20 seminars about strategies for clinical studies for healthcare practitioners at The Jikei University. In 2015, small-group study courses targeting postgraduate students were started from the principles of epidemiology and biostatistics by reading textbooks and by analyzing real clinical data with STATA software (StataCorp LP, College Station, TX, USA) and designing clinical studies. Our goal is for postgraduate students to develop the skills to construct hypotheses, design protocols, monitor trials, and analyze data.

Original studies

1. Randomized trial of vitamin D supplement
2. Elective class of Global Health
3. Randomized trial to prevent food allergy

Publications

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Research Center for Medical Sciences
Division of Clinical Epidemiology

Masato Matsushima, *Professor and Director*

General Summary

The Division of Clinical Epidemiology is promoting the activity of clinical research and clinical epidemiology and of education concerning them. Our specific aim is to support clinicians to solve their own problems in daily practice by epidemiological and clinical research skills.

The research themes of our division are medical communication, quality assessment of medical care, behavioral medicine, outcome research, qualitative research, and disease-oriented epidemiological research. In particular, in the field of primary care we aim to produce evidence owing to the lack of evidence, although primary care is a frontline of practice.

As a contribution to undergraduate education, our division holds classes on evidence-based clinical practice to help turn medical students into skillful physicians who are able to employ an evidence-based approach.

Our postgraduate education concentrates on the methodology of clinical and epidemiological research and biostatistics. "The educational program for primary care on clinical research methodology," which was started in 2007 with the financial support of the Ministry of Education, Culture, Sports, Science and Technology in Japan, was renewed as "The Jikei Clinical Research Program for Primary-care" in 2009. Furthermore, as a subprogram of the project "New Paradigms: Establishing Centers for Fostering Medical Researchers of the Future," supported financially by the Ministry of Education, Culture, Sports, Science and Technology in Japan, "Community Health and Primary Care Medicine" was launched in the doctoral course in 2014. The main aim of these programs is to turn primary-care physicians into clinician researchers.

Research Activities

The EMPOWER-JAPAN study: Elderly Mortality Patients Observed Within the Existing Residence

Little is known concerning the prognosis of patients receiving home medical care in Japan. The EMPOWER-JAPAN study was started as a multicentered prospective cohort study to describe in-home mortality and to clarify its predictors. The cohort consisted of patients who had been newly introduced to home medical care at more than 10 teaching clinics in Tokyo, Kanagawa, and Saitama. The follow-up period was until January 31, 2017. This study was financially supported by the Japan Society for the Promotion of Science.

Development of Japanese version of Patient Centered Assessment Method

With the aging population and with increases in single households and in poverty caused by a disparate society, opportunities to respond to patients with complex problems in the bio-psycho-social aspect are expected to increase in the primary care setting. Preparing a scale to evaluate patient complexity is important. In this research, we plan to develop a Japanese version of the Patient Centered Assessment Method to evaluate patient complexity. The procedures done were forward translation, reverse translation, checking by the original author, and cognitive debriefing. After data collection at family medicine clinics, data analyses were started to evaluate the validity and reliability of the scale.

Relationship between drinking habit and patient complexity: a cross-sectional study at a remote island

As the aging progresses, not only the frequency of multimorbidity, but also social and psychological problems may increase; therefore, understanding of the complexity of patients from bio-psycho-social perspective is indispensable.

Alcohol consumption, on the other hand, causes various social problems such as restrictions on medical access as well as biomedical problems. If we can clarify the relationship between alcohol problems and the scale to evaluate patient complexity, we think that we can clarify a part of what approach should be taken on the bio-psycho-social aspects of patients with alcohol problems.

The aim of this study is to clarify, by means of a cross-sectional survey on a remote island, the relationship of problem drinking measured with the Alcohol Use Disorders Identification Test to patient complexity measured with the Patient Centered Assessment Method.

Education about lesbian, gay, bisexual, and transgender people at medical schools in Japan: a cross-sectional study

Lesbian, gay, bisexual, and transgender (LGBT) people are reportedly exposed to various risks in terms of bio-psycho-social aspects. A systematic review revealed that the knowledge and attitudes of medical staff, such as physicians and students, improved with education about LGBT.

Therefore, in this research, we conducted a questionnaire survey to clarify the current state of time and educational contents spent on education about LGBT at medical schools throughout Japan. Moreover, by comparing our survey data with the present situation in Canada and the United States, we plan to find problems in education about LGBT at medical schools in Japan.

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Research Center for Medical Sciences
Division of Regenerative Medicine

Hirotaka James Okano, *Professor and Director*

General Summary

Regenerative medicine is rapidly moving towards being translated to clinical medicine. However, for regenerative medicine to succeed, the molecular pathways that lead to human diseases must be better understood. To better understand the pathophysiology of neurodegenerative diseases, key roles with be played by studies with good animal models. On the other hand, to study the mechanisms of disease in human cells, differentiated cells of various types can generated and expanded from patient-derived cells via induced pluripotent stem cell (iPSC) technology; these differentiated cells can also be applied to cell therapy. Advances in disease modeling using cells derived from human patients and other primates will have great effects on future opportunities and progress in biomedical research.

Research Activities

Embryonic lethal and abnormal vision gene-like protein 3 regulates neuronal polarity through the alternative splicing

The importance of RNA metabolism in the process of neuronal differentiation and maturation has been widely reported. The RNA-binding proteins modulate RNA metabolism by interacting with target RNAs and regulating the stability, translation, and alternative splicing of RNAs. Neuronal embryonic lethal and abnormal vision gene (*Elav)*-like (Elavl) proteins, composed of Elavl2, Elavl3, and Elavl4, have 3 RNA recognition motifs. A previous study has shown that the long form of Elavl4 localizes in the cytosol and predominantly regulates the stability of RNAs. In contrast, the short form of Elavl4 localizes in the cytosol and nucleus and regulates the alternative splicing of RNAs. The Elavl3 knockout mice exhibited slowly progressive motor deficits and late-onset cerebellar ataxia, and axons of Elavl3 knockout Purkinje cells were swollen (spheroid formation), after which the synaptic formation of axonal terminals was disrupted. A deficit of axonal transport and abnormalities in neuronal polarity were observed in Elavl3 knockout Purkinje cells. We found that the alternative splicing of ankyrin-G (AnkG) exon 34 was misregulated in the cerebella of Elavl3 knockout mice. AnkG localizes at the axon initial segment and nodes of Ranvier in neurons and assembles voltage-gated sodium channels, IV-spectrin, neurofascin, and other related proteins. The clustering of AnkG at the axon initial segment generates the molecular-size-restricted diffusion barrier and substrate-selective filter between the soma and axon and then maintains neuronal polarity. Our study demonstrated that exon 34 of AnkG is alternatively spliced during neuronal development and that misregulation of the alternative splicing of this exon would affect neuronal function. Purkinje cells of Elavl3$^{-/-}$ mice have unique characteristics, and neurons

expressing the “embryonic form” of AnkG, containing exon 34, are observed even in the mature brain. Recently, the existence of immature neurons in adult brains and the abnormal activities of these neurons have been implicated in several psychological disorders. The study of Elavl3$^{-/-}$ mice might also contribute to a better understanding of the relationship between the existence of immature neurons and neurological disorders (Ogawa Y. et al. *Neurosci Res.* 2018).

Cell biological study of an Okinawan large pedigree with bipolar disorder and recurrent depressive disorder

Because bipolar disorder is a psychiatric disease characterized by high genetic heterogeneity, its pathophysiology remains unclear. Despite a genetic component to bipolar disorder, the genomic variations that strongly and directly contribute to this disease have not been specified. A research group of Ryukyu University, our collaborator, found a large family with an autosomal dominant inheritance pattern of bipolar disorder and recurrent depressive disorder in an isolated island of Okinawa. The researchers obtained informed consent from participants and collected blood samples of 8 affected and 8 unaffected individuals. Detected with parametric linkage analysis in a chromosome region was a significant linkage peak, which previous studies have repeatedly reported as a link to bipolar disorder and depression. Whole genomic sequencing-based haplotype phasing determined that a rare haplotype in the chromosomal linkage region was shared among affected individuals of the pedigree. Furthermore, we generated iPSC lines from 3 affected and 4 unaffected individuals of the pedigree and differentiated iPSCs into neurons. An investigation of the phenotype of the iPSC-derived neurons is underway and is expected to uncover the relationship between the genomic variant and the neuronal phenotype.

Primates brain imaging repository for comparative neuroscience

High-resolution magnetic resonance imaging (MRI) and computational analysis technology enable various primate brains to be compared in a 3-dimensional electronic format and to provide precious information about common features across primates and species-specific features in neuroanatomy. To facilitate scientific discoveries in the field of comparative neuroanatomy and brain evolution, we launched a collaborative project to develop as an open resource a repository of nonhuman primate brain images with *ex vivo* MRI. In an initial attempt, we released a collection of structural MRI and diffusion tensor images obtained from 12 species: pygmy marmoset, owl monkey, white-fronted capuchin, crab-eating macaque, Japanese macaque, bonnet macaque, toque macaque, Sykes’ monkey, red-tailed monkey, Schmidt’s guenon, De Brazza’s guenon, and Lar gibbon. Sixteen postmortem brain samples from the 12 species, stored in the Japan Monkey Centre (JMC), were scanned with a 9.4-T MRI scanner of The Jikei University and made available through the JMC collaborate research program (http://www.j-monkey.jp/bir/index.html). The JMC Primates Brain Imaging Repository would contribute as a resource for comparative neuroscience research, an optimizing method to scan large fixed brains, and a reference for veterinary neuroradiology and would preserve various primate brains, including those of endangered species, in a permanent digital form (Sakai T. et al. *Primates.* 2018).

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Research Center for Medical Sciences
Division of Innovation for
Medical Information Technology

Hiroyuki Takao, *Associate Professor and Director*

General Summary

This course deals broadly with information and communication technology (ICT), an area that has recently seen remarkable development, including everything from basic research on its development to clinical application, with the aim of using ICT in medical care.
We are studying the development of wearable devices and artificial intelligence that link with telecommunications. We are also conducting research and development toward implementing ICT medical care in a wide variety of areas, including health management, emergency care sites, intrahospital networks, and chronic-phase rehabilitation and nursing care.

Research Activities

Research and development of a communication application for medical personnel
We are researching and developing a software program called "Join," the first such software to be covered by insurance in Japan. The research investigates factors, including the cost-effectiveness provided by communication in the field of stroke treatment, in which the time leading to diagnosis and treatment is especially important.

Research and development of a health support application
We are researching and developing a software application called "MySOS." When an emergency occurs, this application seeks help from nearby people and helps make the decision whether to go to a hospital, referring to emergency manuals for adults and children. Future development will focus on enabling linkage with hospitals.

Internet of Things development (such as checking blood pressure with a smartphone)
We are going forward with the development of Internet of Things wearable devices as a means of accumulating large quantities of data. In the development of wristwatch-type blood pressure meters and band-type electroencephalograms, we are advancing development from the standpoint of storing large amounts of personal medical information in the cloud via smartphones, and defending against illness.

Mobile phone electromagnetic wave effects
We are doing research related to the effects of smartphones on medical equipment. The research will determine whether there are issues with using smartphones at medical care facilities. We are publishing a paper on this subject.

Medical equipment development (such as intracranial stents)
We are conducting discussions on the development of medical equipment and the practical development of intracranial stents. Currently, the Japanese medical equipment industry is heavily dependent on imports. Our ultimate goal is to contribute to the advancement of the domestic health care industry by offering various types of support and holding physician-led clinical trials, so that the health care industry in Japan can be self-sufficient.

Introducing ICT medical care
We are doing various studies on the introduction of ICT medical care. Using ICT in various aspects of nursing and caregiving might improve work efficiency. The aim is to put ICT medical care into practice.

Medical results of using robots
We are conducting research, using the robot Pepper (SoftBank Robotics), on interaction between robots and people. We are studying what changes occur in health care facilities when people see and come into contact with robots.

Research Center for Medical Sciences
Core Research Facilities for Basic Science
(Division of Molecular Genetics)

Mayumi Tamari, *Professor*
Yuji Ohno, *Assistant Professor*
Yumi Kanegae, *Associate Professor*
Tomomitsu Hirota, *Assistant Professor*

General Summary

Recent advances in technologies and study designs have unveiled the genetic components of human diseases. The aim of our project is to explore genetic factors of allergic and immunological diseases. Interdisciplinary research is necessary to identify the molecular targets and improve our understanding of diseases.
Gene therapy has become an attractive procedure to cure diseases. We contribute to gene therapy by developing methods of regulating gene expression and of genome editing.
We also maintain the following experimental devices, which are commonly used: next-generation sequencing systems, 3130XL sequencer (Applied Biosystems, Life Technologies, Carlsbad, CA, USA), MoFlo XDP cell sorter (Beckman Coulter, Fullerton, CA, USA), flow cytometer, X-ray irradiation research system, and the quantitative real-time polymerase chain reaction. We also support experiments with these devices.

Research Activities

Genetics of inflammatory diseases
Psoriasis is an inflammatory skin disease histologically characterized by epidermal hyperplasia, inflammatory cell infiltration, and vascular changes. A dysregulated cutaneous immune response occurs in genetically susceptible individuals. We have collaborated with Osaka University and Nippon Medical University for researching inflammatory skin diseases. We have recruited patients with psoriasis and conducted an association study of psoriasis and of loci for psoriasis discovered with a genome-wide association study (GWAS). We also perform GWASs, next-generation sequencing analysis, and metabolome analysis of psoriasis.
Recent GWASs have identified genetic variants of the thymic stromal lymphopoietin (*TSLP*) locus which are associated with susceptibility to asthma- and allergy-related phenotypes. We conducted an association study of chronic rhinosinusitis with nasal polyps and aspirin-exacerbated respiratory disease using *TSLP* variants and observed a significant association of rs1837253 with these diseases. Our functional study with a super-shift binding assay suggested an allele-specific influence of rs1837253 on affinity for transcription factors, upstream stimulatory factors (USF) 1 and 2 in nasal fibroblasts. The manuscript containing those findings is under submission.
Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a severe food allergy that usually develops after the ingestion of wheat products followed by physical exercise.

Hydrolyzed wheat gluten protein (HWP) is used as an additive for facial soap. Most patients seemed to be sensitized to an HWP (Glupearl 19S® (Katayama Chemical, INC.) through the use of the facial soap "Cha-no-shizuku (Yuuka Co., Ltd)." Glupearl 19S® is a degraded gluten made from the direct resolution of wheat by hydrochloric acid. We conducted a GWAS including 464 patients with WDEIA induced by HWP-containing facial soap and 3,099 control subjects. Single nucleotide polymorphisms at a region on chromosome 6 were associated with WDEIA induced by HWP-containing facial soap. The manuscript containing these findings is under submission.

An effective strategy for the research of allergic and immunological diseases

Professor Tamari has served as a principal investigator of a group established to make plans for the next 10 years of allergy and clinical immunology research. This work is supported by Health Science Research Grants from the Ministry of Health, Welfare and Labour of Japan. We compiled a report on an effective strategy for research on allergic and immunological diseases and made a brochure explaining the research plans.

Development of the adenovirus vector systems

Because adenovirus vectors (AdVs) are attractive tools for expressing genes and regulating expression, they are applied to many areas of research. The AdVs are also a useful tool to transduce the purpose gene in hepatocytes. We have developed a protocol for curing hepatitis B virus (HBV) infection with an AdV. We established the efficient detection system of HBV genome replication applying AdVs (HBV103-AdV system). We performed high-throughput screening of anti-HBV drugs using this system. As a result, we identified several promising compounds and analyzed what mechanism showed the antiviral effect Furthermore, we succeeded in efficient cleavage of the HBV genome using CRISPR/Cas9 and have developed a hepatocyte-specific genome editing system. In addition, we have constructed AdVs for repairing the beta-glucuronidase (*GUSB*) gene of Sly's disease by genome editing.

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Research Center for Medical Sciences Core Research Facilities for Basic Science (Division of Molecular Cell Biology)

Yoshinobu Manome, *Professor and Director*
Takeo Iwanoto, *Professor*
Keiichi Ikeda, *Assistant Professor*
Akihito Tsubota, *Professor*
Toshiaki Tachibana, *Professor*
Kouki Fujioka, *Assistant Professor*

General Summary

Core Research Facilities for Basic Sciences (Division of Molecular Cell Biology) was established on April 1, 2014. The mission of our facilities is the facilitation of research in the university. Two systems are constituted for the use of our facilities.

1. Annual Registration System

This system is intended to supply research benches and other equipment to researchers of the university to perform experiments. Once registered, researchers can freely use the various devices in our institution. This system also provides, if necessary, technical advice and guidance on specific fine-morphological or biochemical approaches to a registrant's experiment. In 2018, 160 researchers registered at our annual registration system, and we provided research support 214 times for electron microscopy and 1 time for laboratory experiments.

2. System for Providing Research Services

Advances in research technologies and equipment enable us to perform more precise and accurate observations of specimens in medical sciences. For researchers who cannot perform experiments owing to limits of time and funds, our staff can prepare samples for scanning electron microscopy and transmission electron microscopy, record images, and perform high-performance liquid chromatography and mass spectrometry. The service fee is minimal because services are limited to the university.

Research Activities

Investigation of the nicotinamide phosphoribosyltransferase as a potential target for treatment of malignant brain tumors

Mutations in isocitrate dehydrogenases 1/2 are involved in the development of brain tumors, and research on treatment by intervention of this pathway is actively in progress. However, such proteins as epithelial cell growth factor receptor and nicotinamide phosphoribosyltransferase (NAMPT) are overexpressed in brain tumors and are considered to be performant and useful targets of treatment. In particular, NAMPT is a rate-limiting step enzyme of mammalian nicotinamide adenine dinucleotide synthesis, and the amounts of messenger RNA of the enzyme are directly correlated with the prognosis of patients. Therefore, modulation of the NAMPT transcripts is useful as an adjunct to radiation therapy and chemotherapy. This year, vectors transcribing short hairpin interference RNA to NAMPT were constructed and cell lines of stably transcribed short hairpin interference

RNA were established. We are using these cell lines to investigate the effects of NAMPT suppression on cell cycles, growth, drug and radio resistance, and colony-formation of malignant brain tumor.

Establishment of diagnostic method for papillary thyroid carcinoma by measuring tumor-associated antigen

We are currently investigating a clinical application of a monoclonal antibody to papillary thyroid carcinoma established by Professor Takeyama of the Department of Surgery of The Jikei University. In thyroid cells, when quiescent, phosphorylated Yes-associated protein (YAP) is anchored in the cytoplasm, and cells do not proliferate because the activities of phosphoinositide 3 kinase and pyruvate dehydrogenase kinase 1 are not sufficient to activate the Hippo pathway. However, malignant thyroid carcinoma SW1,736 cells have been shown to dephosphorylate YAP and translocate to the nucleus, even in the cells are confluent or in a serum-deprived condition or both. Because the antigen recognized by the monoclonal antibody is glycosylated and localize on the cell surface, the relationship of the antigen with the turbulence of the Hippo pathway is being investigated with cell fractionations and measurements of the quantities of total YAP and phosphorylated YAP.

Characteristics of palmitoyl protein thioesterase 1 and tripeptidyl peptidase 1 enzymes in dried blood spots and leukocytes from patients with neuronal ceroid lipofuscinosis 1 or 2 and their application to newborn screening

We first characterized the enzymes palmitoyl protein thioesterase 1 (PPT1) and tripeptidyl peptidase 1 (TPP1) in dried blood spots (DBSs), plasma/serum, and leukocytes/lymphocytes from patients with neuronal ceroid lipofuscinosis (NCL) 1 or 2 and from control subjects. The enzyme PPT1 had only 1 acid form in control DBSs, plasma/serum, and leukocytes/lymphocytes and showed deficient activities in these samples from patients with NCL 1. In contrast, TPP1 in control DBSs and leukocytes/lymphocytes consisted of 2 forms, an acidic form and a neutral form, whereas serum TPP1 had only a neutral form. In control subjects, the optimal pH of PPT1 in DBSs, plasma/serum, and leukocytes/lymphocytes was 4.5 to 5.0 in the acidic form, whereas the optimal pH of TPP1 was 4.5 in control DBSs and 6.5 in leukocytes/lymphocytes. Regarding samples from patients with NCL 1 or 2, both PPT1 and TPP1 activities in DBSs, plasma, and leukocytes/lymphocytes were markedly reduced in acidic pH, whereas those from heterozygotes with NCL 1 or 2 in the acidic form showed activities intermediate between those of patients and control subjects. In neutral conditions, pH 6.0, the PPT1 activities in patients with NCL 1 showed higher residual activities and intermediate activities in heterozygotes in NCL 1, which was probably caused by mutated proteins in 3 patients with NCL 1. The activities of TPP1 at neutral pH 6.5 to 7.0 in DBSs and leukocytes/lymphocytes were higher in patients with NCL 2 and in heterozygotes. The reason for the increases of neutral TPP1 activities at pH 6.5 to 7.0 in NCL 2 DBSs and leukocytes/lymphocytes is obscure, but the increases might have been caused by secondary activation of neutral TPP1 due to the absence of the acidic form. Interestingly, TPP1 activity in serum consisted of only a neutral form, no an acidic form, and was not deficient in any patient with NCL 2. Therefore,

we can diagnose NCL 1 in patients via a plasma/serum enzyme assay for PPT1, but we cannot diagnose NCL 2 via a serum TPP1 enzyme assay. A pilot study of neonatal screening of NCL 1 and 2 has been established by assays of DBSs from more than 1,000 neonates. Using this assay system, we will be able to perform neonatal screening for NCL 1 and 2 via DBS assays.

Platelets play an essential role in murine lung development through C-type lectin-like receptor 2/podoplanin interaction

Platelets participate in thrombosis, hemostasis, and other pathophysiological processes, including tumor metastasis and inflammation. However, the putative role of platelets in the development of solid organs has not yet been described. Here, we report that platelets regulate lung development through the interaction between the platelet-activation receptor, C-type lectin-like receptor-2 (Clec-2; encoded by the C-type lectin domain family 1, member b gene [*Clec1b*]) and its ligand, podoplanin, a membrane protein. Deletion of Clec-2 in mouse platelets led to lung malformation, which caused respiratory failure and neonatal lethality. In these embryos, alveolar duct myofibroblasts positive for α-smooth muscle actin were almost completely absent in the primary alveolar septa, which resulted in a loss of alveolar elastic fibers and in lung malformation. Our data suggest that the lack of alveolar duct myofibroblasts is due to the abnormal differentiation of lung mesothelial cells, the major progenitors of alveolar duct myofibroblasts. In the developing lung, podoplanin expression is detected in alveolar epithelial cells, lung mesothelial cells, and lymphatic endothelial cells. Lymphatic endothelial cell-specific podoplanin knockout mice showed neonatal lethality and Clec1b2/2-like lung developmental abnormalities. Notably, these Clec1b2/2-like lung abnormalities were also observed after thrombocytopenia or transforming growth factor β depletion in fetuses. We propose that the interaction of Clec-2 on platelets and podoplanin on lymphatic endothelial cells stimulates alveolar duct myofibroblast differentiation of lung mesothelial cells through transforming growth factor β signaling and, thus, regulates normal lung development.

Human hepatocyte chimeric mice and an animal model of hepatitis virus infection

We have established human hepatocyte chimeric mice with an efficient method that we had developed and then used the mice to establish an animal model of infection with hepatitis B virus (HBV) or hepatitis C virus (HCV). We are now investigating the efficacy of novel antiviral agents, the mechanism of progression to chronic infection, and ultrastructural alterations of intrahepatocellular organelles after viral eradication.

Single nucleotide polymorphisms, and resistant-associated variants in the treatment of chronic HCV infection

Direct-acting antiviral agents are the first-line treatment for chronic HCV infection. We are investigating the association of single nucleotide polymorphisms of genes with the blood drug concentration, treatment response, and direct-acting antiviral agent-induced liver damage. Resistant-associated variants are also being investigated in detail.

The association between serum microRNA expression levels and treatment outcome/prognosis in hepatocellular carcinoma

We measure serum microRNA expression levels in intrahepatic feeding arteries, proper hepatic arteries, and peripheral veins when we perform transarterial chemoembolization for patients with hepatocellular carcinoma (HCC) and are investigating the association between serum microRNA expression levels and prognosis/outcome of treatment in patients with HCC who have been treated with transarterial chemoembolization plus radiofrequency ablation.

Intrahepatic cellular localization of ATPase copper transporter beta

The protein ATPase copper transporter beta (ATP7B), also known as Wilson disease protein, is a copper-transporting P-type ATPase that is encoded by the ATPase copper transporting beta gene (*ATP7B*). This protein is located in the trans-Golgi network of the liver and balances the copper level by excreting excess copper into bile and plasma. However, the exact location of ATP7B in hepatocytes is controversial and remains to be determined. We have been cooperating with the seminal research of the University of Barcelona (Spain) and have achieved successful outcomes.

Comprehensive gene expression profiling analysis of microRNA/messenger RNA

We are profiling and analyzing the expression of microRNA/messenger (m) RNA in the liver tissue of HBV-infected human hepatocyte chimeric mice. We have found novel interactions between microRNA and messenger RNA in HBV replication and lifecycle. We are also investigating the association between the serum microRNA expression level and the prognosis/outcome of treatment in patients with HCC who have been treated with transarterial chemoembolization plus radiofrequency ablation.

Identification of cellular secretory pathway of urocortin 2 in HL-1 cardiomyocytes

Because the secretory pathway of urocortins (Ucns), which are members of the corticotropin-releasing factor family of peptides, is only partially understood, we attempted to identify the pathway(s) of Ucn. After the construction of plasmids expressing hybrid proteins of fluorescent protein and Ucn I or II and the instruction of these plasmids into HL-1 cardiomyocytes natively expressing Ucn I and II, the cells were subjected to live cell imaging to track fluorescent proteins with or without brefeldin A, which affects the intracellular dynamics of Ucn I in A172 human glioblastoma cells. We found that the cellular dynamics of Ucn II was not affected by brefeldin A, although that of Ucn I was affected, as previously described.

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Research Center for Medical Sciences Laboratory Animal Facilities

Hirotaka Kanuka, *Professor and Director* Tatsuya Sakurai, *Assistant Professor*

General Summary

The purpose of the Laboratory Animal Facilities is to support *in-vivo* research and to contribute to the development of basic and clinical medicine. In 2018, 806 researchers were registered as users of the Laboratory Animal Facilities. We undertake breeding of experimental animals and provide technical guidance to researchers in animal experimentation. In addition, we performed the following studies to develop basic medical sciences, including laboratory animal science.

Research Activities

Studies of parasite-vector and parasite-host interactions of African trypanosomes
African trypanosomiasis is a deadly protozoan disease of humans and animals. The disease is caused by African trypanosomes, which are transmitted by tsetse flies (*Glossina* spp.). Because the parasites evade host immunity by continuous antigenic variation of their surface coats, all attempts to develop vaccines against the parasites have been hampered. The parasites undergo lifecycle development involving cell differentiation, which is believed to be a promising target for developing novel control measures of the disease. However, the molecular mechanisms underlying cell differentiation are unknown.
We are studying the molecular mechanisms of differentiation from the tsetse fly stage to the mammalian stage in *Trypanosoma congolense,* the lifecycle development of which is reproducible *in vitro*. Using this system, the variation of the parasite's protein expression level during the differentiation from metacyclic forms (tsetse fly stage) to bloodstream forms (mammalian stage) was investigated with liquid chromatography/tandem mass spectrometry analyses. The results show that the expression levels of several proteins thought to be involved in important biological processes, such as iron uptake and mitochondrial electron transport chain, are changed. In addition, the expression levels of several unknown proteins were shown to be altered. Through the functional analyses of these unknown proteins, we are attempting to identify parasite molecules involved in its cell differentiation.

Study on postoperative nausea and vomiting in common marmosets
Common marmosets (*Callithrix jacchus*) are unique laboratory animals. They are primates but are small and have high sociality and good reproductive efficiency. Marmosets often vomit as a complication of anesthesia during induction or awakening. Because vomiting when a patient is half-awake triggers fatal conditions, such as aspiration pneumonia, a reliable method of control is needed. Therefore, we searched for risk factors for postoperative nausea and vomiting (PONV) in marmosets by multivariate analysis. We

found that the incidence of PONV significantly was greater if the patient was young or female or had received general anesthesia, isoflurane, or long-term anesthesia. The presence of multiple factors further increased the incidence of PONV. We are now considering methods to control vomiting through the use of several antiemetic agents with different mechanisms.

Preventing malaria by adjusting amino acid intake

Novel preventive and therapeutic methods against malaria, a major parasitic disease, need to be established because of the emergences of multiple drug-resistant *Plasmodium* strains. Malaria is caused by *Plasmodium* parasites, and these parasites are incapable of most types of amino acid biosynthesis, depending on a part of the amino acid source on free amino acids in plasma. For the better understanding of host-*Plasmodium* interactions, we focused on the amino acids of host plasma and performed "amniography," which is the multivariate index analysis using statistical modeling of the free amino acid composition of blood. In a murine model of cerebral malaria, which is a severe clinical manifestation of the malaria, we have shown that aminogram modification, by adjusting amino acid intake with an isoleucine-deficient diet, prolonged survival in the mice without inhibiting parasite proliferation (cerebral malaria tolerance). Interestingly, analysis with Evans blue of the permeability of the blood-brain barrier of mice indicated no significant difference in the severity of cerebral malaria between control mice and mice fed a isoleucine-deficient diet. However, with magnetic resonance imaging, no inflammatory lesion in olfactory bulb was observed in mice fed an isoleucine-deficient diet. These results indicated the possibility that novel amino acid-mediated mechanisms are involved in the progression of cerebral malaria. Currently, in an *in-vivo* murine model, we are studying the effect of isoleucine deficiency on blood cell morphology and the severity of cerebral malaria.

Publications

Sombié A*[1]*, Saiki E, Yaméogo F*[1]*, Sakurai T, Shirozu T*[2]*, Fukumoto S*[2]*, Sanon A*[1]*, Weetman D*[3]*, McCall PJ*[3]*, Kanuka H, Badolo A*[1] *([1]Université Ouaga 1 JKZ, [2]Obihiro University of Agriculture and Veterinary Medicine, [3]Liverpool School of Tropical Medicine). High frequencies of F1534C and V1016I kdr mutations and association with pyrethroid resistance in Aedes aegypti from Somgandé (Ouagadougou), Burkina Faso. *Trop Med Health.* 2019; **47.** doi: 10.1186/s41182-018-0134-5.

Research Center for Medical Sciences
Radioisotope Research Facilities

Hiroya Ojiri, *Professor and Director*
Haruka Minowa, *Assistant Professor*
Tadashi Asakura, *Professor*

General Summary

The Radioisotope Research Facilities were established to support medical and biological research using radioisotopes. The Facilities also accept the research using nonradioactive isotopes. We have supported researchers by suggesting methods and practical techniques for experiments. Lectures and training courses are held for researchers and for medical students and graduate students. In 2018, the laboratory of this facility was used by 38 researchers from 12 departments and 12 students of 2 curriculums. Major nuclides used for experiments were ^{32}P, ^{51}Cr, ^{125}I, ^{14}C, and ^{3}H. Education related to radiation is also an interest.

Research Activities

Proteasome-resistant cells showed cancer stem cells by highly expressing CD44 and induced epithelial-mesenchymal transition

Down-regulation of E-cadherin plays an important role in epithelial-mesenchymal transition. Our previous study has demonstrated that suppression of E-cadherin induced expression of the transcriptional repressor zinc finger E-box-binding homeobox 1 (ZEB1) in endometrial carcinoma Ishikawa cells resistant to the proteasome inhibitor epoxomicin (EXM-resistant Ishikawa cells) established in our laboratory. Because we found that CD44, a cancer stem cell marker, was expressed in EXM-resistant Ishikawa cells but not in nonresistant Ishikawa cells, we studied the participation of CD44 in the suppression of E-cadherin expression in EXM-resistant Ishikawa cells. The CD44 expressed in EXM-resistant Ishikawa cells was identified as variant 4 (CD44v4). Overexpression of CD44v4 in Ishikawa cells by CD44v4/pcDNA3 transfection induced ZEB1 expression following E-cadherin suppression. On the other hand, CD44v4 suppression (knock-down) in EXM-resistant Ishikawa cells by antisense-CD44v4/pcDNA3 transfection induced E-cadherin expression and ZEB1 suppression. These results suggest that CD44v4 appears in EXM-resistant Ishikawa cells because ZEB1 is upregulated after E-cadherin is suppressed. Moreover, CD44v4 overexpression in Ishikawa cells acquired epoxomicin-resistance, and CD44v4 suppression in EXM-resistant Ishikawa cells repossessed epoxomicin-sensitivity. Epithelial-mesenchymal transition induction via extracellular signal-regulated kinase (ERK) 1/2 signal transduction has been observed previously. Therefore, we will further study CD44 involvement in epithelial-mesenchymal transition induction through ERK1/2 activation/phosphorylation.

Target chemotherapy against CD147-expressed carcinoma cells using polymeric micelles
Because a specific accumulation and cytotoxicity was observed in CD147-expressing cells treated with glutathione-doxorubicin conjugate-encapsulated anti-CD147 antibody-labeling micelles, we prepared tumor-bearing mice to investigate the *in vivo* chemotherapeutic effect.

Chemotherapy for drug-resistant cancer cells with curcumin
Curcumin, a component of turmeric, suppresses nuclear factor kappa B (NF-κB) activation by inhibiting the phosphorylation of inhibitor κB (IκB) bound to NF-κB and inhibiting IκB degradation by curcumin's proteasome inhibitory action. Furthermore, curcumin has been reported to exert an antitumor effect via NF-κB suppression against various types of cancer, including pancreatic cancer and lung cancer, in which KRAS proto-oncogene, GTPase (*KRAS*)-NF-κB is constitutively activated. In addition, colon cancer cell lines were shown to be sensitive to oxaliplatin, depending on the presence or absence of mutations of *KRAS* and *p53*, which do not affect the antitumor effect of curcumin. On the other hand, curcumin is considered to be a sensitive parent to EXM-resistant Ishikawa cells, an adriamycin-resistant, and a cisplatin-resistant ovarian cancer cell line A2780. Curcumin showed equal antitumor activity and the ability to be an effective therapeutic agent for cancers resistant to anticancer drugs.
In addition, curcumin has low bioavailability, and, free curcumin, when taken orally, is metabolized upon absorption from the intestinal tract to reduce its antitumor activity, and an sufficient antitumor effect cannot be obtained. Therefore, the newly developed water-soluble curcumin prodrug — curcumin monoglucuronide — has succeeded in achieving a blood concentration of free curcumin more than 1000 times that of conventional curcumin and is expected to be effective as a novel anticancer agent.

Analysis of resistance mechanisms in radiation-resistant organisms
Tardigrades, which are called water bears, can tolerate extreme environments, including ionizing radiation and dryness. The sludge water bear *Isohypsibius* was isolated from the activated sludge in the Morigasaki Water Reclamation Center, and the terrestrial water bear *Milnesium tardigradum* was isolated from moss collected in Tokyo's Minato Ward. To clarify the radiation-resistant mechanism, tardigrades were irradiated with X-ray at 500 Gy, and DNA damage was analyzed with the comet assay method.

Measuring and tracing of radioactive fallout in the environment
The distribution and behavior of radioactive fallout released into the environment by the accident of the Fukushima Daiichi Nuclear Power Plant in March 2011 have been investigated. Because contaminated water had been leaked into the ocean by accident, we recently examined a safe, simple, and rapid method of analyzing radioactive strontium in seawater. Radioactive strontium was separated with a column of cation exchange resin (Dowex 50WX8, Dow Chemical Company, Midland, MI, USA) and was measured with a newly developed plastic scintillator bottle and a liquid scintillation system (LSC-LB7, Hitachi Ltd.). With this method, the chemical separation of 10 hours (total, 2 days) could be evaluated and compared with 2 weeks via a conventional technique. The detection

limit in this procedure from 1 L of seawater was 0.02 Bq/L. This method might be able to be used to survey contaminated seawater.

Publications

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Research Center for Medical Sciences GMP Production Facilities for Cell Therapy and Gene Therapy

Sadamu Homma, *Professor and Director* Tomoko Ohmae, *Assistant Professor*

General Summary

This facility was established for clinical studies based on cell therapy, gene therapy, and regenerative medicine. Cell products are generated here on the standard of Good Manufacturing Practice for safe administration to the patients of clinical studies. Specified regulation and education have been performed strictly for maintenance of the Good Manufacturing Practice standard in this facility.

Research Activities

Two cell therapy projects — a dendritic cell vaccine against malignant gliomas and a regenerative medicine using a nasal epithelial cell sheet in pearl tumor treatment — have been performed with this cell processing center under the regulation of a new law, the Regenerative Medicine Safety Assurance Act. A new facility for the cell processing center is now being built on the 6th floor of the new hospital building for outpatients. The design of the new cell processing center has been completed this year. The present facility will be closed when the new facility is opened in 2020.

Research Center for Medical Sciences
Institute for High Dimensional Medical Imaging

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

General Summary

The goal of our research is to develop new imaging systems that can be applied to clinical medicine now and in the future. High-dimensional, i.e., 3-dimensional (3D) and 4-dimensional (4D), imaging techniques have enabled noninvasive, realistic, uninhibited, and accurate observations of human spatial structures and their dynamics. The availability of real-time imaging with high-performance computers and medical virtual reality systems has expanded the possibilities for diagnosis, treatment, surgery, and medical education. The Institute for High Dimensional Medical Imaging has, therefore, established a system that facilitates cooperative research and development with international researchers and organizations.

Research Activities

Clinical application of high-definition, real-time medical imaging
We are performing research on the development of medical high-definition imaging technology and its clinical application using functional and morphological data obtained with X-ray computed tomography (CT) and magnetic resonance imaging (MRI).
We are developing a 4D motion system for analyzing human activities, such as the motions of the whole body. The system is driven by motion data obtained from anatomical and skeletal muscle models reconstructed from X-ray CT data sets. Continuing from last year, we measured with MRI the upper and lower limbs of a subject in motion and compared the 4D changes of the skeletal muscle with that of a 4D human model in development. We also evaluated these comparisons. In addition, we have started developing a method to stably measure with MRI the internal structure of humans that change over months and years.

Development of endoscopic surgical robot system
We are developing an endoscopic surgical robot system that can be used to perform natural orifice transluminal endoscopic surgery (NOTES). Robotic instruments enter the abdominal cavity orally and are used to perform surgery on the abdominal organs. In the project to develop an overtube to control the posture of a robot in the abdominal cavity, we are developing a drive mechanism with a shape memory alloy so that the needed power and speed can be obtained for a robot to hold its posture and change directions.

Development of a surgical simulator for various surgical techniques
We are developing a simulator that can deal with various types of surgery, such as laparotomy and endoscopic surgery, using preoperative X-ray CT data of a patient. Last year

we started to develop a simulation system that reproduces the trajectory of surgical instruments and evaluates surgical techniques. This year we started evaluation testing with phantoms and are analyzing the differences in techniques depending on whether the operator has experience. With regards to a 4D image display system for real space, which is a research subject supported by a Japan Society for the Promotion of Science Grant-In-Aid for Scientific Research (A) in its second year of development, we are evaluating and identifying problems using an experimental machine to improve the system.

Development of an image-guided surgery system
We are developing a system that can display blood vessels and tumors at the back of the surgical field in the form of 3D geometric models in multiple layers on the surgical field screen. Such improvements will make the navigation system more intuitive. This year the Department of Surgery again jointly performed navigation surgery in the high-technology navigation operating room of Daisan Hospital as a semiroutine procedure. This year we have developed a new function of the system which warns the operator via voice and images how far the surgical instrument's tip is located from the resected surface planned before the operation. We evaluated the function with a phantom. In addition, in laparoscopic surgery for gynecology, we continue to develop a navigation system that does not use preoperative X-ray CT or MRI data.

Application of high-definition medical image analysis to forensic medicine
By applying technology that we have developed for analyzing high-definition medical images, we are analyzing X-ray CT data sets of crime victims with the aim of developing new methods for future criminal investigations and for establishing new methods for creating court documents. Last year, at the request of the Ministry of the Environment, we started to analyze the cause of death of nationally protected species of animals in traffic accidents. Therefore, we are also developing an analysis method with X-ray CT data obtained from the animals after the accident.

Publications

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Research Center for Medical Sciences
Institute of Clinical Medicine and Research

Toya Ohashi, *Professor and Director*
Ayako M. Watabe, *Professor*
Takashi Sasaki, *Professor*
Midori Kono, *Assistant Professor*

General Summary

The research group run by Professor Sasaki (applied molecular medicine) continued to work on regenerative medicine for pancreatic islets. The group also collected and accumulated clinical samples to find novel volatile biomarkers for inflammatory and metabolic diseases in humans and performed multivariate analysis with a gas chromatography technique. The research group run by Professor Watabe (molecular and behavioral neurosciences) focuses on the neuronal mechanisms regulating aversive and affective memory formation and adaptive behaviors, using molecular, cellular, electrophysiological, and behavioral techniques.
In addition to performing our own research activities, we continue to engage in an educational laboratory course program with the assignment of third-year students from the School of Medicine. We also fulfill research support duties for registered researchers from the University Hospital at Kashiwa campus so that physician-researchers can work efficiently.

Research Activities

Mechanism of islet injury and beta cell regeneration in diabetes mellitus
Pancreatic islet β cells have a unique function to secrete insulin depending on the blood glucose concentration (glucose-stimulated insulin secretion, GSIS). Under *in vivo* circumstances, this function is finely regulated by the nervous system, the microcirculation system, and hormones and metabolites, whereas the failure of this function causes type 2 diabetes. Furthermore, insulinoma, in which regulatory functions such as GSIS are also lost, shows inappropriate hypersecretion. To identify abnormalities of the system of insulin secretion, in a project of the current fiscal year approved by The Jikei University Ethics Committee, we extracted genomic DNA, total RNA, and protein components from the surgical specimens of insulinoma tumor tissue. In this study, we referred to the genome from peripheral blood nucleated cells as the germline of the same person (a patient with insulinoma). When genomes were analyzed in the germline (1,650 million reads, 248 billion bases) and in insulinomas (1,920 million reads, 287.9 billion bases) and compared with the international standard University of California at Santa Cruz reference sequence human genome 19 generic annotation file (UCSC hg19), mutations of 1.3 million blood cells and insulinomas were found. When analysis was limited to high-precision reads of the sequences, 540,000 sites (hereinafter referred to as "PASS") were found. Of the PASS, 67 genes were found to be mutated in insulinomas but not in the germline, and 92 genes were mutated in blood cells but not in insulinomas. Furthermore, of the mutations in

PASS, 90,787 were in the exon region, of which 41 were definitely pathogenic and 7 were likely pathogenic (they differed from UCSC hg19 in both the germline and insulinomas, suggesting that these originated from the germline genome). A study of whether these 48 exonal variations are responsible for the dysregulation of insulin secretion in insulinoma cells could lead to a better understanding of insulin secretion failure in diseases, including diabetes. This research was supported by a Grant-in-Aid for Scientific Research, with Professor Sasaki as the principal researcher.

Search with gas chromatography for novel volatile biomarkers in breath and skin gas
Substances derived from volatile organic compounds (VOCs) are detected as 400 or more types of volatile components in human breath, and many of them are attributable to metabolism in the body. The types of VOCs may differ depending on the pathological condition, and detection of these differences can be applied to early, noninvasive diagnosis and preemptive medicine. The VOCs exhaled by patients with inflammatory conditions were analyzed in a research study in collaboration with Professor Masami Nemoto of The Jikei Katsushika Medical Center and with the approval of the Ethics Committee of this university. Gas was analyzed with gas chromatography/mass spectrometry in collaboration with Professor Takeo Iwamoto (Division of Molecular Cell Biology), and VOCs that were quantitatively and qualitatively different from those of healthy people were searched for. The detected VOCs were identified with a mass spectrum database compiled by the National Institute of Standards and Technology, and multivariate analysis was performed of associations with diseases. The results so far have clearly shown a specific pattern of VOCs (a profile formed by a plurality of VOCs) in patients with inflammatory diseases. In the future, we will consider using artificial intelligence to identify the specific pattern for each disease. This research was performed at The Jikei University, where Professor Sasaki was the research leader, and with the support of the pioneering joint research promotion fund.

Elucidating the circuitry mechanisms underlying fear memory learning
Avoiding pain and harm is fundamental for the survival of humans and other animals. Aversive stimuli, therefore, potently induce adaptive behaviors and memory formation. Clarifying neuronal circuitry mechanisms underlying such adaptive behaviors is fundamental to understanding brain functions. Furthermore, the dysregulation of the neuronal circuitry of such aversive behaviors leads to various anxiety disorders, such as posttraumatic stress disorder, and other psychiatric diseases.
The amygdala is acknowledged as a critical brain region to attach the aversive valence of nociceptive stimuli onto various sensory stimuli. This association is considered to be mediated *via* synaptic plasticity, which underlies certain forms of learning paradigm, such as fear conditioning. Although neuronal networks and plasticity mechanisms for fear conditioning have been intensively studied, not much is known about how the emotional value of pain itself is regulated at the circuitry level.
In previous studies, we have identified one such nociceptive pathway: neurons in parabrachial nuclei (PB) of the pons form a direct monosynaptic projection on the central amygdala (CeA). We found that the PB-CeA pathway is necessary and sufficient for fear mem-

ory formation, suggesting that the PB–CeA pathway might be involved in some emotional aspects of pain.

Our research in 2018, with real-time conditioned place avoidance in a Y-maze test, found that the optogenetic activation of the PB–CeA pathway is perceived by mice as an aversive signal (in preparation for publication). Also, we have reported in a review article that the PB serves as an integration site for multimodal information, including pain, hunger, taste, and general metabolism; therefore, synaptic plasticity at the PB–CeA pathway might contribute to the modification of the emotional valence of sensory information (Nagase et al., Curr Opin Behav Neurosci., 2019). We have contributed to a study of lysosomal storage disease conducted by Professor Ohashi (Division of Gene Therapy, Department of Pediatrics).

We found that a mouse model of MGII with LSD knock-out exhibited impaired fear memory formation and that cell-targeted gene therapy with strong preconditioning significantly improved the phenotype to a level comparable to that of wild-type mice (in preparation). These works were supported by a Grant-in-Aid for Scientific Research (C), the Strategic Research Program, and Core Research for Evolutional Science and Technology to Professor Watabe and by the Agency for Medical Research and Development and a Grant-in-Aid for Scientific Research (B) to Professor Ohashi.

Publications

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Centers of Advanced Medicine
Center for Neuroscience of Pain

Fusao Kato, *Professor and Director*

General Summary

The Jikei Center for Neuroscience of Pain (JCNP) was established in April 2014 as the first member of the Core Centers for Advanced Medicine of The Jikei University as a stronghold to advance the clinical and biomedical research on various aspects of the neuroscience of pain, under the support of the Ministry of Education, Culture, Sports, Science and Technology (MEXT)–Supported Program for the Strategic Research Foundation at Private Universities (S1311009; FY2013–2017).

After concluding the 5-year project in FY2017, the JCNP has continued its activity in FY2018 as a central site to develop and advance clinical and nonclinical studies and to teach about the neuroscience of pain.

The JCNP has been a center for collaboration between pain researchers and graduate students belonging to clinical or nonclinical units. These units include the Departments/Divisions of Neuroscience, Gene Therapy, Regenerative Medicine, Institute of Clinical Research, Anesthesiology, Orthopaedic Surgery, Rheumatology, Diabetes, Metabolism and Endocrinology, Dermatology, Obstetrics and Gynecology, and Pharmacology. In addition to performing collaborative research activities, we have also organized joint seminars, colloquiums, and open talks.

Research Activities

The JCNP has submitted the final report for the MEXT-Supported Program for the Strategic Research Foundation at Private Universities (No. S1311009) to the MEXT, which is posted on the MEXT website:
http://www.mext.go.jp/component/a_menu/education/detail/__icsFiles/afieldfile/2018/11/16/1410554_012.pdf

Centers of Advanced Medicine
Center for Medical Entomology

Hirotaka Kanuka, *Professor*
Kenji Ishiwata, *Professor*
Tatsuya Sakurai, *Assistant Professor*

General Summary

Arthropod vectors are organisms that play a role in the transmission of pathogens from humans or animals to humans. Vectors tend to be blood-sucking insects that ingest the disease-causing organism with blood from an infected host and then insert the organism into a new host during their next blood-meal. A new strategy to control vectors should absolutely be developed and involved in integrated vector management, because such a strategy would be an extremely effective means of dealing with the problem while waiting for a vaccine or another effective dengue control strategy. In our center, based on our collaboration with institutions in endemic countries, such as Burkina Faso, Nigeria, and Taiwan, entomological studies promoting multilateral approaches have been performed to gather knowledge of the diagnosis, ethology, immunity, and epidemiology of vector species on effective vector control.

Research Activities

Vector control strategies utilizing the symbiotic bacteria Wolbachia

Symbiotic microorganisms prevail in huge varieties of insect species, supporting insect adaptation to diverse habitats based mainly on nutritious interactions. *Wolbachia* are the most prevalent endosymbiotic bacteria in invertebrates and are estimated to be infecting more than 60% of insect species. *Wolbachia* are transmitted vertically through host eggs and manipulate their hosts in a variety of ways: cytoplasmic incompatibility (CI), male killing, male-to-female transformation, and parthenogenesis. In particular, CI is the most prominent phenomenon induced by a variety of *Wolbachia* strains, causing embryonic lethality when infected males mate with uninfected females. Another fascinating feature of *Wolbachia* bacteria is their ability to induce positive-stranded RNA virus resistance in insect host cells. *Aedes* mosquitoes infected with *Wolbachia*, especially when introduced from other insects, have extremely lower levels of viruses after feeding on blood containing Zika, dengue, or yellow fever viruses and resultantly become incompetent to transmit them to mammalian hosts. Recently, promising practical approaches using *Wolbachia* have emerged to control *Aedes* populations in current or potential risk areas of dengue or Zika. Two distinct strategies have been adopted on the basis of the separate features implemented on *Aedes* mosquitoes by *Wolbachia* infection: male sterility by CI and virus blocking. Field releases of *Wolbachia*-infected male mosquitoes are expected to effectively reduce population size via CI. *Wolbachia*-infected females otherwise replace the current natural population with a virus-incompetent population after generations. Our research is now focusing on revealing molecular bases of the *Wolbachia*-host interactions

and, concomitantly, the effect of *Wolbachia* on insect evolution and ecology. Our histochemical and biochemical analyses have revealed that *Wolbachia* associate closely with Dengue virus genomic RNAs and hamper amplification of Dengue single-round infectious particles, which indicate that replication of viruses can be prohibited by *Wolbachia*. The maternal ribonucleoprotein complexes associated with *Wolbachia* in host female germline cells accumulated at the Dengue virus replication machinery, supporting the hypothesis that the *Wolbachia*-ribonucleoprotein interaction is also the causal element of the RNA virus blocking by *Wolbachia*.

Dissecting overwintering mechanism of Asian tiger mosquito, Aedes albopictus

Aedes albopictus is an *Aedes* species widely distributed from East Asia to India. In the tropic zone, the *A. albopictus* mosquito repeats its life cycle throughout the year. On the other hand, in temperate zones, such as Japan, when adult females of this species are exposed to short days and low temperatures in late autumn, they lay diapause eggs. In diapause eggs, development is paused in the stage of pharate first instar larvae. The arrest of hatching behavior is maintained until early summer, and the pharate larvae show strong resistance to coldness, drying, and starvation. To reveal the genetic mechanisms of preparation and maintenance of diapause in *A. albopictus*, we focused on 2 strains: a tropic strain and a temperate strain. We confirmed that a Kuala Lumpur strain and a Hiroshima strain showed different hatching rates when adult females were reared with short days and low temperatures. Furthermore, we compared temporal and comprehensive gene expression between eggs from the tropic strain and diapause eggs from the temperate strain using RNA sequencing to identify genes involved in diapause mechanism. Our results have shown several candidate genes involved in environmental resistance and have also suggested the possibility that the neuropeptide gene Capability (*Capa*) induces hatching behavior. We are now attempting to establish *Capa* mutants to evaluate the function of Capa protein in the formation and maintenance of diapause eggs.

Publications

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Centers of Advanced Medicine
Center for Medical Science of Fatigue

Hiroyuki Yanagisawa, *Professor and Director*

General Summary

The Jikei Center for Medical Science of Fatigue (JCMSF) was established in 2014 with support from the Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Strategic Research Foundation at Private Universities. The JCMSF is aimed at contributing to human welfare through developing novel methods for the diagnosis, prevention, and care of fatigue-related diseases. For this aim, our research focuses on the mechanisms of fatigue and fatigue-related diseases.

Resulting from stress or insomnia — whether related to work or not — fatigue is something that everyone experiences. Long-term fatigue can cause cardiovascular dysfunction, such mental disorders as depression, and occupational sudden death (*karoshi*). Fatigue is, therefore, a major social problem. Physiological fatigue can be recovered from with rest. In contrast, pathological fatigue persists for 3 months or more and greatly affects quality of life. Because pathological fatigue requires therapeutic interventions, it must be distinguished from physiological fatigue. Levels of human herpesvirus (HHV)-6 and HHV-7 DNA in saliva increase with training and decrease with rest, suggesting their usefulness as biomarkers of physiological fatigue and cancer-related fatigue (CRF).

Research Activities

Clinical significance of CRF in patients with multiple myeloma

An adverse event in patients with multiple myeloma — treated with cytotoxic agents, proteasome inhibitors, and such immunomodulatory drugs as bortezomib, lenalidomide, and thalidomide — is CRF. The aims of our study were to prospectively analyze the clinical significance of CRF and to evaluate the cumulative incidence of CRF and the survival rates of 16 patients with multiple myeloma who were treated with proteasome inhibitors and immunomodulatory drugs. Reactivation of salivary HHV-6 and HHV-7 was analyzed with the real-time quantitative polymerase chain reaction. The CRF was evaluated with a visual analog scale. The subjects of this study were 11 patients with newly diagnosed multiple myeloma and 5 patients with relapsed or refractory multiple myeloma. The cumulative incidence of CRF was 54.9%. The incidence of CRF was not associated with the type of treatment. The cumulative incidence of reactivation for HHV-6 was 73.1% and for HHV-7 was 45.6%. However, the reactivation of HHV-6 and HHV-7 was not related to CRF. Overall survival and progression-free survival among patients with newly diagnosed multiple myeloma were significantly shorter for those with CRF than for those without CRF. In conclusion, CRF is a major symptom in patients with multiple myeloma and predicts shorter overall survival and progression-free survival in patients with a new diagnosis.

Attenuation of human herpesvirus 6B reactivation by aging

Objective: Little research has evaluated HHV-6B infection in healthy adults, and the prevalence rates in different age groups have remained unclear. Therefore, the major objectives of this study were to evaluate the seroprevalence of HHV-6 antibodies in working people and to examine the effect of aging on seroprevalence. Also, because HHV-6B is reactivated in saliva, another objective was to investigate an association between age and HHV-6B reactivation based on measured salivary levels of HHV-6 DNA.

Methods: Our subjects were 77 ordinary office workers who underwent a health checkup. In this population, we measured anti-HHV-6 antibody titers with enzyme-linked immunosorbent assay and measured salivary HHV-6 DNA levels. In addition to examining an association with age, we examined associations with body mass index, smoking habit, and alcohol consumption as confounding factors.

Results: The seropositivity of HHV-6 antibodies decreased significantly in subjects 50 years and older, and age was significantly negatively correlated with anti-HHV-6 antibody titers. Age and salivary HHV-6 DNA levels were also significantly negatively correlated, but no significant correlations with other factors was found.

Conclusions: Our results suggest that HHV-6B reactivation is attenuated by aging. Thus, HHV-6 antibodies steadily decrease in the body with aging.

Centers of Advanced Medicine
Stable Isotope Medical Application Center

Tomokazu Matsuura, *Professor*
Takashi Okano, *Professor*
Koji Nakada, *Professor*
Takeo Iwamoto, *Professor*
Koji Takada, *Professor*
Youichiro Kusakari, *Associate Professor*

General Summary

The Spontaneously Diabetic Torii (SDT)-Fatty rat model of diabetes was used to accumulate data until an age of 40 weeks using the fasting ^{13}C-glucose breath test on the onset of liver insulin resistance.
For clinical research, we promoted practical use of liver insulin resistance evaluation with the fasting ^{13}C-glucose breath test for patients with cardiovascular disease.

Publications

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Centers of Advanced Medicine
Center for Biofilm Science and Technology

Yuki Kinjo, *Professor and Director*
Katsuhiko Yanaga, *Professor*
Shoichi Uezono, *Professor*
Takeo Iwamoto, *Professor*
Ken Kaito, *Professor*
Shinya Sugimoto, *Associate Professor*
Tadayuki Iwase, *Associate Professor*
Ken-ichi Okuda, *Assistant Professor*
Ryuichi Nagahori, *Assistant Professor*
Seiji Hori, *Professor*
Keishi Marumo, *Professor*
Shin Egawa, *Professor*
Koji Takada, *Professor*
Tetsuya Horino, *Associate Professor*
Jun Araya, *Associate Professor*
Akiko Tajima, *Assistant Professor*
Noriyuki Murai, *Assistant Professor*
Midori Kono, *Assistant Professor*

General Summary

The Jikei Center for Biofilm Science & Technology (JCBST) was established in April 2015 as a member of the Centers of Advanced Medicine of The Jikei University with the support of the Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Strategic Research Foundation at Private Universities.
Biofilms are intricate communities of microbes that form on biotic and abiotic surfaces. Within biofilms, microbes are embedded in a typically self-produced extracellular matrix composed of proteins, polysaccharides, and DNA which provides microbes survival advantages in stressful environments. Thus, biofilms formed on the surfaces of medical devices and tissues can often cause what are known as chronic biofilm-associated infections. The JCBST, based on collaboration with basic and clinical research laboratories, aims to promote research for understanding molecular mechanisms of biofilm formation and for preventing and controlling biofilm-associated infections.

Research Activities

Detection of bacterial DNA from central venous catheter removed from patients by next generation sequencing
Catheter-related infection (CRI) is a serious challenge in clinical practice. This study examined how well next-generation sequencing (NGS) targeting 16S ribosomal DNA is able, compared with the culture method, to identify causative pathogens in CRI. The genera identified with NGS were consistent with those from conventional culture tests. The culture method and NGS showed high agreement, and, in patients suspected to have CRI, had a sensitivity of 80.0% and a specificity of 76.9%; meanwhile, the false-positive rate of NGS was as low as 4.0% in patients who were judged as having no infection symptom by the physicians and whose catheter was removed because it was no longer needed. In conclusion, NGS targeting 16S ribosomal DNA was able to analyze the bacterial composition of central venous catheter specimens and detect causative pathogens in patients with CRI and was, therefore, suggested as a promising diagnostic tool for CRI.

Mechanisms of RNA stabilization in staphylococcal biofilms

Staphylococcus aureus often causes serious infections, such as biofilm-associated infections due to formation of robust biofilm. Understanding the molecular mechanisms of how biofilms form is important to develop new strategies against these infections. We have previously found that RNA is a component of the biofilm formed by MR10, which is a clinically isolated strain of methicillin-resistant *S. aureus* and forms a robust biofilm in an extracellular polysaccharide-dependent manner. Although RNA is generally recognized to be unstable in the environment, how it is stably retained in the MR10 biofilm is unclear. To address this question, interaction between RNA and polysaccharides was analyzed in the MR10 biofilm and in vitro. Biochemical, microscopic, and molecular interaction analyses demonstrated that RNA directly interacts with and co-localizes with polysaccharides in the biofilm. Additionally, genome sequence analysis suggested that *nucA*, encoding extracellular thermonuclease with DNase and RNase activities, is completely deleted in MR10. As expected, the plasmid-born intact *nucA* from the strain Newman abolished biofilm formation of MR10. These results suggest that production of extracellular polysaccharides and deletion of *nucA* are key for the retention of RNA in the biofilm at an extremely high level.

Role of extracellular adherence protein in establishment of rugged and thick structures of S. aureus *biofilms*

MR23, a clinically isolated strain of methicillin-resistant *S. aureus*, forms a biofilm containing a large amount of secreted extracellular adherence protein (Eap). So far, we have found that (1) Eap and the cell-wall-anchoring protein *S. aureus* surface protein G (SasG) work redundantly to form an extensive biofilm, (2) cell-wall-anchoring is required for SasG to promote biofilm formation, (3) SasG binds DNA and protects it from degradation, and (4) simultaneous deletion of the genes *eap* and *sasG* significantly reduce mortality against silkworms. In this study, we analyzed the roles of Eap and SasG for determining the 3-dimensional structure of the biofilm. Confocal laser scanning microscopy with thioflavin T-staining of biofilms revealed that deletion of *eap* reduced the roughness but not the thickness of the biofilm, whereas the deletion of *sasG* did neither. In addition, double knockout of *eap* and *sasG* decreased both roughness and thickness. These results suggest that Eap and SasG work redundantly in terms of the amount of the biofilm, while only Eap contributes to the complicated biofilm structures. These findings indicate the importance of simultaneously analyzing multiple molecules, which could lead to a deeper understanding of the mechanisms of biofilm formation.

Imaging of biofilms in solution by atmospheric scanning electron microscopy

In this study, we visualized aqueous biofilms formed by the Gram-positive coccus *S. aureus* and the Gram-negative bacillus *Escherichia coli* by means of recently developed atmospheric scanning electron microscopy (ASEM). Membrane vesicles, delicate spiral flagella, straight curli fibres, and filamentous extracellular DNA networks were observed with ASEM and labelling methods, such as labelling with positively charged Nanogold and heavy metals. In addition, surface adherence of *Paracoccus* sp. and *Leptothrix* sp. was analysed with ASEM. Collectively, our results suggest that ASEM is a broadly appli-

cable approach for microbial research and diagnostic purposes.

Quality control of the type 8 secretion system in E. coli

The *E. coli* type 8 secretion system is involved in the secretion and formation of extracellular Curli amyloid fibers. We found that Curli biogenesis depends on the molecular chaperone DnaK, a bacterial Hsp70 homolog, via a quantity and quality control of RpoS, a stationary phase-specific alternative sigma factor regulating bacterial transcription, and CsgD, the master transcriptional regulator of Curli formation. DnaK also keeps CsgA and CsgB in a translocation-competent state by binding to their signal peptides prone to aggregation. We also found that certain periplasmic chaperones and proteases cooperatively maintain the quality and quantity of CsgA and CsgB in the periplasmic space. In addition, we identified epigallocatechin gallate as an effective inhibitor of Curli biogenesis. These results provide mechanistic insights into Curli biogenesis and robust biofilm formation.

Publications

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Clinical Research Support Center

Shigeru Kageyama, *Professor and Director*
Shinji Yasuno, *Associate Professor*
Sho Takahashi, *Assistant Professor*
Masako Nishikawa, *Professor*
Minoru Chida, *Associate Professor*

General Summary

The Clinical Research Support Center was founded in April 2014 to promote the proper conduct of clinical research. The center has the following functions: protocol planning, statistical analysis, data management, monitoring, support for clinical research conduct, and education. We started consulting for clinical research in September 2014 and had 64 protocols of consultation from April 2018 through March 2019. The number of protocols the center consulted on was as follows: research planning, 16; protocol planning and statistics, 25; protocol for randomization/allocation/concealment of emergency key, 4; statistical analyses, 24; preparation of articles, 10; response to reviewers after the submission of articles (including additional analyses), 5; application for the Agency for Medical Research and Development or the Ministry of Education, Culture, Sports, Science and Technology, 10; transition to specified clinical research, 5; and conducting statistical analysis, 7. Consultations were requested by the Departments of Endoscopy, Otorhinolaryngology, Biochemistry, Urology, Neurosurgery, Clinical Oncology/Hematology, Surgery, Orthopaedic Surgery, Anesthesiology, Rehabilitation Medicine, Psychiatry, Cardiovascular Surgery, Pediatrics, Radiology, Breast Endocrinology Surgery, Obstetrics and Gynecology, Pathology, and Emergency Medicine; the Research Center for Medical Sciences; and by the Divisions of Nephrology and Hypertension, Digestive Surgery, Gastroenterology and Hepatology, Regenerative Medicine, Neurology, Respiratory Diseases, and Diabetes, Metabolism and Endocrinology.

In cooperation with the Division of Clinical Pharmacology and Therapeutics we held a "Clinical Trial Seminar" to improve literacy about clinical trials among researchers. The theme was "Big data in cardiovascular diseases and the Clinical Innovation Network" (October 2018). We also held a "Biostatistics Seminar for Tomorrow" consisting of 2 basic courses and 1 advanced course to promote appropriate trial designs and the application of biostatistical methods.

Ethical guidelines for medical and health research involving human subjects have been implemented since April 2015. In addition, the Clinical Trials Act has been enforced since April 2018, after which a certified review board was established at The Jikei University. To meet these requirements, we prepared common forms of protocol, an informed consent form, a standard operating procedure for monitoring, and other documents. For monitoring, clinical research coordinators supported investigators to conduct clinical trials properly.

We introduced a clinical research liaison system to facilitate clinical research. We requested departments that are conducting many clinical trials to assign liaison physicians. Liaison physicians are invited to participate with priority in the "Biostatistics Seminar for Tomorrow" and are expected to act as liaisons between departments to which they

belong and the Clinical Research Support Center.
As a measure against disasters, a clinical data extraction system from electronic health records was introduced in the 4 affiliated hospitals of The Jikei University by using the Standardized Structured Medical record Information eXchange (SS-MIX). We have started to establish a disease registry based on this system in cooperation with medical departments.

Research Activities

Owing to the nature of our center, we collaborate with researchers to conduct various types of clinical studies. In cooperation with the Division of Diabetes, Metabolism and Endocrinology, Department of Internal Medicine, we showed that HbA1c, blood pressure, the body mass index, body weight, and levels of high-density and low-density lipoprotein cholesterol have certain circannual rhythms in patients with type 2 diabetes. Evaluating the variability or absolute value of HbA1c, taking circannual rhythms into consideration, will likely improve the accuracy and precision of risk prediction. However, measuring HbA1c every month in general practice seems difficult because of medical costs.
In our research, we designed population pharmacodynamics models in patients with type 2 diabetes with sparse sampling data to reflect circannual rhythms under real world conditions. As part of the Japan Diabetes Clinical Data Management Study Group, we used patient records from scientific activities to create and validate a model.

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Hospital, [11]***Tokai Univ,*** [12]***Hamamatsu Univ,*** [13]***Mie Univ*** [14]***Kobe Red Cross Hospital,*** [15]***Hiroshima Univ,*** [16]***Nagasaki Rosai Hospital,*** [17]***Chubu Rosai Hospital,*** [18]***Tohoku Univ,*** [19]***Gifu Univ,*** [20]***Yamaguchi Univ).*** Study protocol for the G-SPIRIT trial: a randomized placebo-controlled, double-blinded phase III trial of granulocyte colony-stimulating factor-mediated neuroprotection for acute spinal cord injury. *BMJ Open.* 2018; **8:** e019083.

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Premedical Course

Biology

Koji Takada, *Professor* Rie Hiratsuka, *Associate Professor*

General Summary

Our research themes are as follows: (1) studies of the evaluation and mechanism of cytotoxicity associated with proteostasis disruption and (2) subcellular localization analysis of endogenous adjuvant molecules in Japanese cedar pollen.

Research Activities

Development and standardization of the cytotoxicity evaluation system by quantification of hardly-soluble polyubiquitin-protein conjugates

Eukaryotic cells have various mechanisms to keep the internal environment stable. The ubiquitin-proteasome system and autophagy are parts of such mechanisms and maintain cellular protein homeostasis (proteostasis) by appropriately degrading unnecessary proteins. Because proteasome recognizes polyubiquitin tags as degradation signals and because the autophagic pathways preferably degrade polyubiquitin-containing aggregates in a ubiquitin adaptor p62-dependent manner, the polyubiquitin amount reflects the state of proteostasis. Exposure of epithelial cells to cadmium at a level equivalent to a median effective concentration (EC_{50}) induces significant increases of hardly-soluble polyubiquitin amounts preceding cell death. Therefore, we regard this phenomenon as cytotoxicity accompanied by proteostasis disruption (proteostatic toxicity) and are attempting to clarify its biological significance. Following last year, we refined a series of toxicity assessment procedures for labor saving. In addition, we tried to standardize the analysis protocols to expand this system to the toxicity analysis of various chemicals. In the first step, the EC_{50} of a test substance for the 48-hour exposure of the cultured cells is determined. In the second step, the same cells exposed to the test substance of the EC_{50} for 24 hours are collected, and "the soluble fraction" extracted with 1% Triton X-100 and "the hardly-soluble fraction" insoluble in 1% Triton X-100 but soluble in 2% sodium dodecylsulfate are prepared. In the final stage, levels of proteins and polyubiquitin in both fractions are assayed, and the case where the amount of the hardly-soluble polyubiquitin significantly increased by the exposure to the EC_{50} was judged as proteostatic toxicity-positive. Examination with this criterion using epithelial cells showed that cadmium, methylmercury, and arsenous acid were strongly positive; that hexavalent chromium was mildly positive; and that paraquat was negative. Molecular mechanisms of proteostatic toxicity will be studied by systematically analyzing various chemical substances.

Localization of β-1, 3-glucan in Japanese cedar pollen

To clarify the adjuvanticity of β-1, 3-D-glucan (β-glucan) contained in Japanese cedar

pollen, its localization in pollen was analyzed immunohistochemically. The results clearly showed that β-glucan was localized in the exine (the outer layer of a pollen wall) and in the generative cell wall. After water absorption, β-glucan localized in the exine was exposed to the outside by its rupture. Co-workers have shown that the exine induces tumor necrosis factor α and interleukin 6 production in a β-glucan-receptor-dependent manner. In the onset and exacerbation of Japanese cedar pollinosis, β-glucan localized in the exine is considered to stimulate the innate immune system in a β-glucan-receptor-dependent manner and to promote the immune response to a pollen allergen.

Physics

Tsuyoshi Ueta, *Professor* Katsumi Kasono, *Assistant Professor*

General Summary

1. We have proposed a disordered air rod photonic crystal as a model of a sponge structure inside a barb of the red-flanked bluetail and are attempting to reproduce the structural color of birds by confirming the reflection spectrum.
2. We have found that an incident electromagnetic wave is amplified resonantly within an artificially vibrating 1-dimensional metallic photonic crystal. We are investigating the relation of the conditions of the amplification to the virtual bound states.
3. We are studying an ultrasonic lens with an adaptively deformable phononic structure constructed with microtubes into which liquid metal is injected. In this research, we are attempting to stimulate a cerebral deep part by designing a phononic lens in which a brain and the cranial bones are also taken into account as metamaterials.
4. We have been studying computational methods and algorithms for the condensed matter theory. The phenomena of interest are phase transitions and critical phenomena.

Research Activities

1. We consider amplification of an incident electric-magnetic wave within a disordered photonic crystal (photonic amorphous), which is made of stacked metallic plates of random thickness arranged in parallel with equi-intervals. We have confirmed that amplification of an incident electromagnetic wave occurs by resonance with weakly localized states, namely the virtual bound states.
2. We propose a computational method of the most suitable initial structure for topology optimization and show the resulting structure (acoustic lens). To generate the phononic structure with multiple focuses, we employ a holographic technique with which a suitable phononic lens is obtained as low-valued domains of the interference pattern between radiative waves from all of the focal points within the skull and a reference wave, namely a plane wave. The phononic structure is the same as the so-called Fresnel zone plate. The influence of scattering by the skull on the phononic structure will be discussed.

3. A challenge is to determine the distribution of the liquid metal so that the ultrasonic wave can be converged to the thrombus accurately and intensively. To this end, we aim to develop a topology optimization system based on the boundary element method, boundary representation with level set function, time evolution of level set function, and the adjoint variable method. To verify our system, we solved a (simplified) optimization problem to maximize the sound pressure at a single point in air, without considering other media, such as the skull and brain. First, we used a box as the fixed design domain, where the liquid metal (scatter) is allowed to appear and disappear. The resulting liquid metal looked like a Fresnel zone plate. The sound pressure markedly increased at the target point.
4. We performed multigrid cluster Monte Carlo simulations of q-state ferromagnetic Potts models on square lattices. We studied relaxation times of parameters and energy.

Publications

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Chemistry

Takashi Okano, *Professor*
Naruyoshi Komiya, *Associate Professor*

General Summary

The research of this laboratory is focused on synthesis-oriented organic chemistry, including the synthesis of bioactive compounds and fluorine-containing materials, and the development of novel functional organic/organometallic compounds for fine organic synthesis.

Research Activities

Preparation of fluorine-containing organic compounds via N-heterocyclic carbene catalysis
N-heterocyclic carbenes (NHCs) are highly stable carbanions stabilized by the aromatic azolium system, including vitamin B_1. These NHCs are now attracting interests as environmentally compatible organic catalysts for various reactions, using aldehydes as acyl anion equivalents. For the application of NHC-catalyzed reactions to the synthesis of biologically active fluorinated materials, the strongly basic reaction conditions would cause a problem. Recently developed precursors for NHCs without basic reaction conditions were used in an attempt to check NHC reactions of fluorinated aldehyde hemiacetals.

Regiospecific remote Pt-H interactions in oligomethylene-vaulted (N^C^N)-pincer Pt(II) complexes

(N^C^N)-Pincer type bis(methylimino)phenylPt(II) complexes having a macrocyclic vaulting structure consisting of deca- undeca-, and dodecamethylene bridges were synthesized and subsequently characterized with nuclear magnetic resonance, infrared, and mass spectrometries and single-crystal X-ray powder diffraction. The unprecedented remote intramolecular Pt-H interactions were observed on the linker hydrogens at β- and ζ-positions with highly regiospecific manner both in crystal and solution states. The hydrogen bonding nature originated from overlap between Pt dz^2 and σ* of the specific C-H bonds was revealed with natural bond orbital analysis on the basis of density functional theory calculations.

Publications

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Social Science (Law)

Ryuichi Ozawa, *Professor*

General Summary

Problems of Constitutional Law in present-day Japan

Research Activities

Ozawa published articles and books cited in the Japanese version of Research Activities 2018.

Human Science

Kazushi Misaki, *Professor*

General Summary

The Study of Western philosophy and ethics

Research Activities

Origin of the ego: The intersubjective approach to the subject
Descartes' "cogito," the ego as the subject of thought, is still a popular and paradigmatic image for the human subject: to be a mature human means that one can think independently and autonomously and can act according to one's own beliefs.
In modern philosophy this image of the ego has been attacked from various positions. From one such position, an intersubjective approach criticizes Descartes' cogito as an isolated subject and maintains that an ego can be a subject only in intersubjective relations. Through the recognition of the other, one can become and can be a subject. Studies by Donald Winnicott have shown how important the relationship of a baby with its mother is at the first stage of the ego. George Herbert Mead considered the development of the ego as a process of "ideal role-taking of others." The goal of this development is a subject that can think from the universal point of view, as Descartes imagined.

Learn from the experience in Auschwitz
From another respect, the "inhuman" situations in the Auschwitz concentration camp show various elements needed to be "human." From the experiences at Auschwitz written about by Viktor Frankl, we can learn the "human conditions" that in ordinary life remain unconscious but essential.

Japanese

Ikuko Noro, *Professor*

General Summary

A study of objective happiness and resilience.

Research Activities

A Web-based survey was administered to 480 workers in Japan to investigate the degree of happiness and resilience they perceive. We found that the degrees of objective happi-

ness and resilience were highest in workers older than 60 years, both male and female, and were lowest in male workers younger than 30 years. We presented the results at the 30th annual meeting of the Japanese Society of Developmental Psychology.

Mathematics

Katsuya Yokoi, *Professor* Yasuko Hasegawa, *Assistant Professor*

General Summary

I. To study dimension theory and topological dynamics
II. Some applications of automorphic forms of several variables to number theory

Research Activities

I. We studied omega-limit sets, (strong) chain recurrent sets on topological dynamics, the Conley index theory, and the Lusternik-Schnirelmann category.
II. We clarified the second term in the Laurent expansion of a real analytic Siegel-Eisenstein series around a certain point. Furthermore, we proved its harmonicity and automorphicity.

English

Osamu Ohara, *Professor* Tetsuro Fujii, *Professor*

General Summary

English audiovisual education and digital medieval English study (Ohara)
English language communication and education: material analysis and development (Fujii)
Ohara continued his study of graphology and morphology in the letters of the Pastons in the 15th century. Ohara also continued an investigation concerning how to make useful digital images and XML files of 15th century manuscripts, especially of the Paston Letters. The results of this investigation were discussed in the papers read at an international conference.
Fujii joined a project team to compile English textbooks for high school English classes: *English Communication I*, *II*, and *III*. Along with compiling the textbooks, Fujii has been writing their exercise materials and teacher's manuals. In addition, Fujii has been studying how teaching materials influence learner motivation and language development.

Research Activities

Ohara presented a paper at sessions of the International Medieval Congress held at Leeds University in England in July and at Aichi Educational University in December.
Fujii analyzed and collected authentic English materials to meet the level and the needs of high school textbooks based on current teaching methods, theories, and research findings on learning English as a foreign language. These materials were used to compile textbooks following the revised teaching guidelines set out by the Ministry of Education, Culture, Sports, Science and Technology. A new edition of the textbook, *World Trek – English Communication III*, was officially approved by the Ministry and published in February 2019.

Reviews and Books

Mochizuki M[1], Aizawa K[2], Allum P[3], Sasabe N[4], Hayashi Y[5], Fujii T, Miura S[6] ([1]Reitaku Univ, [2]Toyo Denki Univ, [3]Rikkyo Univ, [4]Toritsu Aoyama High, [5]Koshigaya High, [6]Tsurubunka Univ). *World Trek English Communication III.* Tokyo: Kirihara Shoten; 2019.

Mochizuki M[1], Aizawa K[2], Allum P[3], Sasabe N[4], Hayashi Y[5], Fujii T, Miura S[6] ([1]Reitaku Univ, [2]Toyo Denki Univ, [3]Rikkyo Univ, [4]Toritsu Aoyama High, [5]Koshigaya High, [6]Tsurubunka Univ). *World Trek English Communication III: Teacher's Book.* Tokyo: Kirihara Shoten; 2019.

First Foreign Languages

Katsumi Suzuki, *Professor*

General Summary

German contemporary literature

Research Activities

My research topic is the modern German literature of nonnative writers in German-speaking areas.
I am working now with the novels of Sherko Fatah. His father is Kurdish-Iraqi, and his mother is Polish-German. In fact, he is a native writer of German with the background of an immigrant. His heroines are always playing their part in Germany, as well as in the Middle East, mainly in Iraq. What is offered to him in the creative work as subjects, is the fatherland of his father. He writes his work in his mother tongue. I have already written and published an essay about the relationship between the fatherland and his mother tongue in his novels.

School of Nursing

Fundamental Nursing

Sachiko Tanaka, *Professor*
Hiroko Yatsu, *Professor*
Sumiko Satake, *Assistant Professor*
Noriko Sato, *Professor*
Chieko Hanyu, *Assistant Professor*
Noriko Aoki, *Assistant Professor*

General Summary

Major study areas in basic nursing include: (1) education on physical assessment and supporting techniques, (2) supporting techniques in daily living, (3) history of nursing, (4) supporting patients with progressive motor dysfunction, and (5) nursing diagnosis.

Research Activities

Sachiko Tanaka studied the healthy work environment of nurses in hospitals.
Hiroko Yatsu published a book with 2 co-authors focusing on qualitative research methods. She is researching global nursing by means of an integrative literature review and is writing books on nursing theory and ecological psychology which will be published in 2020.
Chieko Hanyu performed a questionnaire survey of teachers to understand the current status of education on physical assessment accompanying the revision of the 2009 curriculum.
Sumiko Satake studies under the theme of "a reply of autonomic nerve activity to hearing stimulation in patients with long-term lying in bed." In addition, she has worked as a research member of neuroscience nursing and positioning in nursing.
Noriko Aoki studied how intra-abdominal pressure is decreased by changing the head elevation angle while the patient uses a bedpan.

Publications

Aoki N. Sense of Learning Achievement for Excretion Assistance Techniques through On-Campus Training and On-Site Clinical Training for Nursing Students. *Nursing and Primary Care.* 2018; **2:** 1-7.

Adult Nursing

Misuzu Nakamura, *Professor*
Nidori Nagano, *Professor*
Miwako Fukuda, *Associate Professor*
Tetsuya Myojin, *Assistant Professor*
Masami Sato, *Professor*
Ruka Mochizuki, *Associate Professor*
Yoko Murooka, *Assistant Professor*
Wakako Osaka, *Assistant Professor*

General Summary

Undergraduate students were offered classroom coursework, including an introduction to clinical nursing and 4 areas of clinical nursing based on the level of health (the chronic phase, the perioperative period, cancer, and the acute phase). An educational evaluation was held with an emphasis on the process of learning practical nursing skills through the chronic phase and on a perioperative nursing practicum. As part of their research activities, the faculty members explored cancer nursing topics and nursing care for acutely ill and critically ill patients.

Research Activities

Research on critical care

1. Development of a support program utilizing reflection to foster nursing practice capabilities in critical care
2. Research on a supporting program for developing practical skills for critical care nursing

The purpose of this study was to develop a program to support the practical capability of nurses working in critical care. Our program comprised 3 monthly sessions consisting of a combination of group reflection and simulation, each lasting for 1 day. We believe that the reflection of a group of nurses working in different environments encourages the rearrangement and rebuilding of pattern recognition and improves metacognition.

Research on the perioperative period

1. A color index of clinical drainage fluid has only recently been established but remains uneven between physicians and nurses because their judgement is based on experience knowing the colors of drainage. We promote the study of the clinical color index of drainage fluid on the basis of its composition, and the color analysis of the blood component in unexplored. The aims of this study are to establish and clinically apply a color index for drainage fluid.

Research on cancer nursing

1. Research on cooperation of pharmacists and nurses for patients with cancer

Our pilot study examined a model of collaboration of pharmacists and nurses for patients who have cancer and family members who have gone to a pharmacy. We conducted semi-structured interviews of 7 pharmacists supporting such patients and their families. The results were presented at an academic conference.

2. Research on chemotherapy-induced peripheral neuropathy
In joint research with other facilities, we have been developing educational applications for patients and the model of a comprehensive multisector care system for chemotherapy-induced peripheral neuropathy. This year, we have conducted randomized controlled trials to evaluate a developed application. In addition, to develop a comprehensive care system model, we conducted qualitative research targeting multiple occupations.
3. Research on the support of patients who have cancer and are raising a child
The purpose of this research was to develop assessment tools and applications for the concerns of patients who have cancer and are raising a child and to develop comprehensive care models based on indicators. This year, we are planning to conduct Internet surveys.

Research on other topics
1. Research on factors related to the quality of life of ostomates
The aim of this study is to describe the characteristics of patients who have a new ostomy.
2. Characteristics of patients with pressure injuries found during hospitalization
This study aimed to describe the characteristics of patients who have pressure injuries during hospitalization.
3. Research on and development of the use of mini-sheets to improve blood flow and prevent pressure ulcers for patients at home
We collected data on changes in contact pressure and blood flow when mini-sheets were used. The subjects were patients of a hospital's convalescent ward who used wheelchairs on a daily basis. The collected data showed an increase in tissue blood flow and a decrease in contact pressure. In the future, we plan to analyze the data and use it to develop new tools.
4. Measuring pressure and shear force on the heel region and the reduction effect using dressing materials
The purpose of this study was to measure the pressure and shear force on the heel when the head is elevated and to determine whether these variables are reduced when dressing materials are used on the heel. The subjects were healthy volunteers 30 years or older. Pressure and shear force were measured by the angle from the lying position to the elevated head position. Subjects were also divided by film dressing, hydrocolloid dressing, and silicone foam dressings, and the pressure and shear force on the skin surface were measured when these 3 types of dressings were applied on the skin surface with a 3-axis tactile sensor. We plan to proceed with the analysis in the future.

Publications

Nagano M, Ogata Y, Ikeda M, Tsukada K, Tokunaga K, Iida S. Peristomal Moisture-Associated Skin Damage and Independence in Pouching System Changes in Persons with New Fecal Ostomie. *J Wound Ostomy Continence Nurs.* 2019; **46:** 137-42.

Gerontological Nursing

Fumiko Kajii, *Professor* Yoshie Nakajima, *Associate Professor*

General Summary

The following 4 studies were conducted in 2018: The first study was of the development and evaluation of a fall-detection application and a support program for elderly persons to monitor fall-prevention methods. This study was supported by a Grant-in-Aid for Scientific Research (B). The second study was of the development of an educational program to train unemployed and retired nurses to support persons who live in the community who have dementia and are cared for by family members. This study was supported by a Grant-in-Aid for Challenging Exploratory Research. The third study, a collaborative study by the city of Komae, The Jikei Daisan Hospital, and The Jikei University, was a survey of the problems of caregivers for family members with dementia. This study was supported by the research expenses of the School of Nursing. The fourth study was of the development of a physiological and psychological quantification method of "soothing sensation" using music-combined rehabilitation. This study was supported by a Grant-in-Aid for Scientific Research (C).

Research Activities

Study 1: The subjects were elderly people 65 years or older to whom a fall-prevention lecture was delivered 3 times every alternate week (intervention group, 21 subjects) or was not delivered (control group, 38 subjects). We collected data on the subjects' mental and physical health conditions and lifestyle during the first lecture, 12 weeks later, and 24 weeks later. After Within 24 weeks, the risks of falling, stumbling, and sliding did not differ significantly between the intervention group (0.38, 0.42, and 0.15) and the control group (0.23, 0.47, and 0.09). The risk of any event with a smartphone was higher for the intervention group than for the control group. Hence, the risk of falling, stumbling or slipping was not reduced by the lecture. However, use of a smartphone increased awareness of fall prevention in daily life. This data is now being analyzed.
Study 2: The aim of the educational program was to train potential nurses to support persons with dementia and their families, on the basis of data obtained in 2016.
Study 3: We clarified the support needs and problems of 166 family caregivers of persons with dementia receiving home service or community-based service by care insurance in home care support establishment in the Komae area.
Study 4: The purpose of our research is to establish a self-management program supporting patients with moderate paresis after stroke to engage in everyday rehabilitation and to achieve physical functional recovery. As a first step, we are attempting to verify the outcome of music combined with rehabilitation and to develop a nursing support program. In a preliminary study with healthy volunteers, we investigated the effect of emotion on movement intention and identified factors that affected movement-related cortical poten-

tial in button-pushing movement. When the music presented in combination with rehabilitation had a clear rhythm, comfortable tonality and melody, and an appropriate volume, the amplitude of the movement-related cortical potential increased, suggesting increased preparation for voluntary movement that might enhance the intention to exercise. On the basis of this result, we plan to investigate the relationship between emotion and motor function in patients with moderate paresis.

Mental Health and Psychiatric Nursing

Yasuko Koyano, *Professor*
Junko Ishikawa, *Assistant Professor*
Mayuko Yamashita, *Associate Professor*

General Summary

We give lectures to teach students about medical systems and social resources based on the mental health and welfare acts. We also give lectures to teach students about methods to assess patients who have mental problems during treatment.

Research Activities

Analysis of the difficulties of service providers of persons who have autistic spectrum disorder but no intellectual disabilities
A self-administered questionnaire survey was conducted on the difficulties of 141 staff members of psychiatry day-care institutions who provide support to adolescents and adults who have autistic spectrum disorder (ASD) but no intellectual disabilities. Analysis of free-text entries of the survey revealed difficulties in the following areas resulting from a lack of knowledge among support providers: "understanding of ASD characteristics," "concrete responses to be taken," and "planning of tailor-made support." In addition, the analysis revealed that, due to the ASD characteristics of these persons, service providers experience "difficulties in resolving issues."

Theory of self-care for people with mental disability in a community
The purpose of this study was clarification of the elements of self-care for people with mental disability in a community. We extracted the constituent elements of self-care for people with mental disabilities in a community. Self-care was composed of six elements of "stability of mental and physical states," "maintenance of daily life," "ability to accept support," "maintenance and development of human relations," "empowerment," and motivation in life," and 28 element divisions and 86 concrete activities. The elements of self-care are useful indexes to ascertain the self-care necessary to realize the life-style and life desired by people with mental disabilities and to design support.

Publications

Koyano Y, Watanabe H. Analysis of the difficulties experienced by service providers supporting adolescents and adults having autistic spectrum disorder without intellectual disabilities — Survey results from 141 psychiatry day — treatment institute. *Bulletin of Juntendo Institute of Mental Health.* 2018; **29:** 55–8.
Yamashita M, Yabuta A, Iseki T. Elements that supporters who take root community support recognize as necessary for people with mental disability to live in a local community. Journal of Japan. *Academy of Psychiatric Mental Health Nursing.* 2018; **27:** 82–90.
Yamashita M. Theory of self-care for people with mental disability in a community. *The Journal of Practical and Professional Nursing.* 2018; **2:** 1–6.

Child Nursing

Kinu Takahashi, *Professor*

Michie Nagayoshi, *Assistant Professor*

General Summary

The lectures given to undergraduates included an introduction, methods, practice, and an educational evaluation. These lectures promoted the learning of practical abilities in pediatrics through training in outpatient clinics and inpatient wards and the neonatal intensive care unit of The Jikei University and Child Development Center. These educational methods were used to enhance the advocacy of children's rights, to promote the mother-child relationship and family-centered care in clinical situations, and to deal with, practice, and learn nursing skills. Through practical training, the students learned about nursing for hospitalizing children with disease, children living in the community at an acute disease stage, family-centered care, and multidisciplinary collaboration.

Research Activities

Development of the ability scales of regarding child advocacy for pediatric nurses: Analysis of reliability and validity

With this study we aimed to develop a scale assessing the ability of pediatric nurses to achieve practices that advocate children's rights and verify its reliability and validity. The draft scales were extracted from previous studies, and their items and validity were examined by teachers and nurses engaged in pediatric nursing and education. Thirty-one items of the draft scales were prepared. Furthermore, an anonymous self-administered questionnaire survey using these items was conducted with 627 pediatric nurses in 30 healthcare facilities in the Kanto region, including 25 university and 5 pediatric hospitals. The effective recovery rate was 58.2%. On the basis of item and factor analyses, 19 items were classified into 3 factors: "ability to understand and support children and their family," "ability to adjust members of the medical staff who did not advocate children's rights," and "ability to explain to the child and confirming his or her own view." Cronbach's α coefficient for the overall reliability was 0.86, and its content validity was presented. However, additional challenges face studying the items of the scales and the criterion-related validity for another enlarged object of the survey. The scales were confirmed

to be practicable if their accuracy were improved.

Development of a scale to improve the ability of pediatric nurses to advocate children's rights

The ability scale was established to improve the ability of pediatric nurses to achieve practices that advocate children's rights, and its reliability and validity were verified. In stage 1, the scale was prepared. In stage 2, the validity of the scale was examined. In stage 3, a pilot test was conducted. As a result of exploratory factor analysis, 19 items were classified into 3 factors. Factor 1 was named "the relation that they try to understand between children and their family," factor 2 was named "the adjustment of members of the medical staff who did not advocate children's rights, and factor 3 was named "an explanation to a child, and the confirmation of his or her will." The Cronbach's α coefficient for the overall reliability was 0.86, and correlation with the existing scale was confirmed. This ability scale was realized to have a certain level of reliability and to have validity from a viewpoint of criterion‐related validity and known‐groups validity. The cumulative contribution rate by factor analysis, however, was 43.25%, and further investigation is required.

This study will be presented at the 28th conference of the Japanese Society of Child Health Nursing.

Difficulties and coping‐action of childhood cancer survivors in their school life after returning to their school

The purpose of this study was to understand the difficulties faced by children who have recovered from cancer, coping, and support in their development after being discharged from the hospital. A qualitative descriptive analysis was performed of data obtained through semistructured interviews with 9 survivors of childhood cancer. Difficulties identified were composed of 37 subcategories and 14 categories based on 83 codes. These problems were related to school life or work. Fourteen categories were composed for coping strategies. This study found that survivors, because of physical weaknesses and problems, had school lives when they re‐enrolled that differed from those of their friends.

This study was presented at the 50th Congress of the International Society of Paediatric Oncology, Kyoto.

A prospective study of relationships between developmental characteristics of 4‐ to 5‐year‐old infants after retinoblastoma treatment and parenting stress

This study aimed to clarify the developmental characteristics of patients with retinoblastoma in early childhood. A prospective study using the Strengths and Difficulties Questionnaire (SDQ) and the Parenting Stress Index (PSI) was conducted at a 6‐month interval of 20 mothers of 4‐ to 5‐year‐old children with retinoblastoma. Twenty dyads were recruited in this study, and all 20 (100%) agreed to participate. No significant differences in PSI and SDQ scores were observed between time points. During the second tests, more mothers showed high‐risk scores on PSI's "Mood," which positively corelated with SDQ's "Conduct problems" ($p < .001$). "More problems/worries" scores were significantly higher than the normative means ($p < .05$). High need scores on SDQ's "Prosocial"

were shown during the first tests by 6 children (30.0%) and during the second tests by 4 children (20.0%).
This study was presented at the 50th Congress of the International Society of Paediatric Oncology, Kyoto.

Roles of fathers of 0- to 3-year-olds in double-income households, and related factors
This study investigated the employment conditions of parents of 0- to 3-year-old children attending nursery school, the role and child-rearing behavior of fathers, and relationships between these factors and child development. A cross-sectional, self-completed questionnaire survey was conducted of 290 fathers, and 57 respondents (19.7%) were analyzed (mean age: 37.2 years [SD = 11.9]). Early Childhood Parenting Scale scores showed significant negative correlations with the PSI child domain ($\rho = -.49$, $p < .001$) and parent domain ($\rho = -.53$, $p < .001$). The state of the father's role correlated with the number of hours worked by mothers per week ($\rho = .30$, $p < .05$). Providing advice and positive feedback on involvement in nurturing to fathers who bring their children to school every day might promote good father-child relationships.
This study was presented at the 16th Congress of the World Association for Infant Mental Health, Rome.

Maternity Nursing

Yasuko Hososaka, *Professor* Mayumi Hamada, *Lecturer*

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

Aspects of the boundary between discipline and abuse by fathers raising infants
We examined the specific content of childcare anxiety and abuse anxiety from the narratives of 11 fathers. From the interview data, 497 codes were extracted and classified into 5 categories. We found that fathers had narrow boundaries between discipline and abuse in childcare and that discipline was likely to lead to abuse. These results suggest the need for assistance to prevent the transition from discipline to abuse.

Creation of a Japanese-language version of the Quality Assessment Tool for Quantitative Studies Ensuring Equivalence and a study of its reliability
In the present study, a Japanese-language version of the Quality Assessment Tool for Quantitative Studies (QATQS), a scale used to comprehensively evaluate quantitative

research from the standpoint of the quality of the research, was created while the equivalence of the 2 versions was ensured and the reliability of the Japanese version of QATQS was demonstrated. The equivalence of the Japanese version of QATQS created in the present study was confirmed by the original authors. The results of the reliability study showed that the interrater reliability of the tool was high and indicate that the effectiveness of published studies can be objectively evaluated.

Mothers' experiences of feeding their infants: a meta-summary of qualitative research

In this study, we integrated the results of qualitative research on the experiences of mothers feeding their infants. Information from 40 qualitative studies published in Japan from 2000 through 2015 was collected to create a meta-summary. The findings were summarized as 30 statements, with the following 9 topics of experience identified: (1) demonstrating the autonomy needed to make the choice as to method of feeding their infant, (2) independently choosing the method of feeding, (3) making efforts to breastfeed, (4) projecting the mother-child relationship onto the act of breastfeeding, (5) projecting oneself as a mother onto giving breast milk, (6) coping with weaning at different rates, (7) being negative about feeding, (8) feeling happy with the support provided, and (9) feeling disappointed with the support provided. The effects with the highest frequency (frequency rate, 20% to 38%) included experiences of physical and mental pain associated with breastfeeding and pumping, questioning of one's self-worth as a mother, and demonstrating maternal autonomy and determination. Further studies should examine problem situations surrounding breastfeeding mothers, such as physical and mental suffering caused by breastfeeding and their self-worth being shaken, and creating appropriate support that respects the autonomy and wishes of mothers.

Literature review of antenatal breastfeeding education designed to alleviate difficulties in breastfeeding

The aim of the present study was to review studies that have examined methods of antenatal breastfeeding education whose goal is to lessen the difficulties that breastfeeding mothers might experience. Previous studies were identified with literature searches of relevant electronic databases (PubMed, the Cumulative Index to Nursing and Allied Health Literature, Cochrane, and ICHUSHI) and hand searches of reference lists from key studies published in English and Japanese. The main reason studies were excluded from review was that participants were not women in advanced countries. This review included data from 10 studies, mostly from developed countries, including the United States, Australia, Singapore, and Japan. At different research levels, peer counselling, lactation consultation, and face-to-face styles of education during pregnancy appear to increase the duration of breastfeeding. However, no conclusive evidence was found to recommend any specific form regarding antenatal breastfeeding education for lessening any difficulties that breastfeeding mothers may experience. Although several studies of antenatal breastfeeding education examined difficulties in breastfeeding, they evaluated mainly the rate of starting and the duration of breastfeeding. In general, the only indicators of difficulty were nipple pain and mastitis. No studies evaluated the negative effects of antenatal breastfeeding education or mothers' satisfaction levels.

Community Health Nursing

Junko Shimasawa, *Professor*
Yumiko Shimizu, *Assistant Professor*
Yoshiko Kubo, *Assistant Professor*

General Summary

The faculty's research has focused on: (1) visiting nursing care to promote continued community life by mentally ill patients living at home, (2) exploring competencies regarding the stress check system among occupational health nurses, (3) the relationship between career anchors and job and home life satisfaction among occupational health nurses (OHNs) in Japan, (4) health and welfare in patients undergoing hemodialysis who live in the community, and (5) nursing intervention for self-care by patients undergoing hemodialysis.

Research Activities

Visiting nursing care for mentally ill patients living at home
The purpose of this study was to clarify the features of assistance provided by visiting nurses to promote the continued community life of mentally ill patients living at home. In this study, such assistance was considered to support and promote the continued life in the community of mentally disabled persons in an individually suitable manner.

Exploring competencies regarding the stress check system among occupational health nurses
This study examined competencies regarding the stress check system by occupational health nurses. Ten occupational health nurses participated in the semistructured interview. Data was analyzed with descriptive qualitative methods.

Relationship of career anchors to job and home life satisfaction among OHNs in Japan
This study examined the relationship of career anchors to job and home life satisfaction among OHNs in Japan. The characteristics of career anchors among OHNs resemble those found in previous studies. The OHNs with more experience in their career have higher job satisfaction, and we suggest that many OHNs should be appointed to managerial positions for career development.

Health and welfare in patients who are undergoing hemodialysis and live in the community
This study aimed to clarify problems of health and welfare in patients who are undergoing hemodialysis and live in the community. We analyzed factors associated with a depressive status among patients undergoing hemodialysis.

Nursing intervention for self-care by patients undergoing hemodialysis
This study aimed to clarify the nursing intervention for self-care by patients undergoing hemodialysis.

Publications

Kubo Y, Hatono Y[1], Kubo T[2], Shimamoto S[3], Nakatani J[4] ([1]Kyushu Univ, [2]National Institute of Occupational Safety and Health, [3]Tokai Univ, [4]Univ of Occupational and Environmental Health). Relationship between Job and Home Life Satisfaction and Demographic Characteristics among Occupational Health Nurses in Japan. *Japanese Journal of Occupational Medicine and Traumatology.* 2018; **66:** 289-97.

Sugisawa H[1], Shimizu Y, Kumagai T[2], Sugisaki H[3], Ohira S[4], Shinoda T[5] ([1]J.F. Oberlin Univ, [2]Osaka City Univ, [3]Hachioji Azumacho Clinic, [4]Sapporo Kita Clinic, [5]Tsukuba International Univ). Barriers to Effective Case Management for Disabled Patients on Hemodialysis. *Therapeutic Apheresis and Dialysis.* 2018; **22:** 133-41. Epub 2017 Dec 28.

Home Care Nursing

Motoko Kita, *Professor*
Yuri Sugiyama, *Assistant Professor*
Hiroko Toyama, *Assistant Professor*

General Summary

Since 2011, our undergraduate course, Home Care Nursing, has focused on students acquiring the ability to develop the nursing process based on the characteristics of home care nursing, by studying home care nursing skills and home care nursing practices, which range from theory to practical training. In fiscal year 2018, we are planning to launch an educational assessment study. Also, in fiscal year 2017 we did research on the areas of interest of each member of the teaching staff.

Research Activities

Recognition by students of home health nursing training regarding the management of patient information and subsequent actions
Owing to the Internet and social networking services, a large amount of nonspecific information can now be easily collected and transmitted. Consequently, the need has increased for students to both recognize and properly perform information management. We are doing research to raise recognition by students of information management during home health nursing training and to obtain indications regarding the instructive relationship so that students can safely perform information management. We plan future surveys of how patient information is recognized by students and is managed.

A study of discharge support program construction for elderly persons with dementia at an acute care hospital
An increasing number of elderly persons with dementia are admitted to acute care hospi-

tals to have other diseases treated, and supporting these patients upon discharge is difficult. A study of multiple cases aims to clarify the discharge-support process for elderly patients with dementia involving nurses of an acute hospital's discharge support division and to develop a discharge support model corresponding to the difficulties of dementia.

Unexpected re-hospitalization

In Japan, as medical expenses continue to increase, needed to reduce hospitalization and treatment costs are appropriate assistance for patients and families during hospital discharge, the avoidance of re-hospitalization, and continuation of a safe and comfortable life with medical treatment. Consequently, through a review of literature in Japan and overseas regarding assistance during hospital discharge to avoid re-hospitalization, we are planning to examine research trends, to clearly identify the contents and effects of specific assistance during hospital discharge and future challenges, and to determine the conditions at acute phase hospitals.

Development of a liaison model for pediatric patients using multiple home-visit nursing service facilities

As the number of children with medical complexity living at home increases, so does the demand for home-visit nursing services. However, at present, few facilities and nurses can provide such services. Because the service facilities of home-visit nursing tend to be of a small scale, cooperation among them might strengthen the systems' support of pediatric patients receiving home care and of their families. Therefore, we are conducting a study to develop a cooperative model of multiple home-visit nursing service facilities for pediatric patients.

Inspection/evaluation

Home Care Nursing has introduced active learning into the class. However, the class must be further improved. We will continue our educational assessment to offer more effective education.

Because all research conducted by our instructors involves important subjects in the field of home care nursing, we must support each other and widely publicize our research findings by writing articles.

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Research Activities 2018

The Jikei University School of Medicine

〔非売品〕

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